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LEVEL OF DIETARY FAT DOES NOT AFFECT FUEL OXIDATION OR ENDURANCE EXERCISE PERFORMANCE OF SOLDIERS

U S ARMY RESEARCH INSTITUTE
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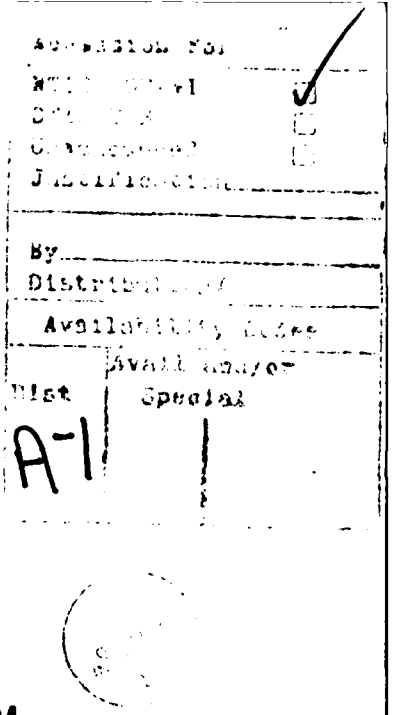

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DIETARY FAT DOES NOT AFFECT FUEL OXIDATION OR ENDURANCE EXERCISE PERFORMANCE OF SOLDIERS

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The objective was to determine whether additional dietary fat calories influenced the physiology or endurance exercise performance of physically active soldiers. Eight male soldiers (age 22±2 yrs, $\bar{X}$±SEM) participated in two five-day test cycles while eating either a basal diet (2300 kcal; 40% fat Calories) alone or with additional fat calories (3300 kcal and 57% fat Calories). Carbohydrate (300 g/day) and protein (70 g/day) levels were kept constant. A four-day exercise program (total daily energy expenditure = 4645±237 kcal/day), was followed by a progressive treadmill test to exhaustion on day five. The exercise endurance times did not differ between the basal diet alone (106.1±11.2 min) or with additional fat calories (106.5±7.6 min). There were no significant physiologic differences between the groups at rest or during exercise. However, changes in resting blood chemistry and gas exchange indicated that fat metabolism predominated regardless of diet. In conclusion, a) the fat content of rations has little short-term effect on either physiologic responses or physical performance of active soldiers, b) short-term fat requirements can be met by using body fat stores, and c) a dietary carbohydrate intake of 300 g/day is insufficient to prevent a transition from a carbohydrate- to a fat-predominant metabolism during four days of moderate exercise.

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EXECUTIVE SUMMARY

The primary test objective was to determine whether additional dietary fat calories influenced the physiology or endurance exercise performance of physically active soldiers. Eight male soldiers (age 22±2 yrs, body wt 80.7 ± 4.5 kg, $\dot{V}O_2max$ 4.08 ± 0.22 L/min) participated in two five-day test cycles while eating either a basal diet (2300 kcal; 40% fat Calories) alone or with additional fat calories (3300 kcal and 57% fat Calories in total diet). Carbohydrate (300 g/day) and protein (70 g/day) levels were kept constant. A four-day exercise program, consisting of three hours per day of regular intermittent moderate exercise resulting in a total energy expenditure of approximately 4645±237 kcal/day, was followed by a progressive treadmill test to exhaustion on day five. Gas exchange was measured at rest and during exercise. Venous blood samples, taken on day 1, day 5, and during exercise, were analyzed for insulin, lactate, glucose, free fatty acids, and β-hydroxybutyrate. The exercise endurance times did not differ between the basal diet alone (106.1±11.2 min, $\bar{X}$±SEM) or with additional fat calories (106.5±7.6 min). There were no significant differences in blood chemistries between the groups at rest or during exercise. However, decreases in resting blood insulin, lactate, and glucose levels, and respiratory exchange ratio, and increases in free fatty acid and β-hydroxybutyrate levels indicate that at this level of work and dietary carbohydrate intake, fat metabolism predominated regardless of diet. In conclusion, a) the fat content of rations has little short-term effect on either physiologic responses or physical performance of moderately active soldiers, b) short-term fat requirements can be readily met by using body fat stores, and c) dietary carbohydrate intake of 300 g/day is insufficient to prevent a transition from a carbohydrate- to a fat-predominant metabolism during four days of moderate exercise.

Recommendations: (1) Set as a development goal for packaged rations the delivery of greater than 300 g (400 to 440 g) of carbohydrate per day in a palatable form. (2) Deliver this amount of carbohydrate in conjunction with 70 g protein and as much fat as practical within weight and volume constraints.

INTRODUCTION

The modern soldier must have special operations, patrol, or assault field rations that are palatable and meet nutrient requirements. These rations also should have minimum mass and volume and tolerate a wide range of storage conditions. These latter constraints, which may reduce the quantity and palatability of the food, have made it difficult for any field ration system to meet all the energy and macronutrient needs of the physically active soldier (1). While a general consensus exists that the protein content of rations should be limited (2), there is disagreement as to whether field rations should include additional fat to increase caloric content or be designed to meet carbohydrate requirements (3-5).

Dietary energy intake in excess of energy expenditure normally leads to increased body fat stores. These neutral fats, which are stored without water, have a high free energy content per unit weight, releasing 9 kcal/g on complete combustion. In humans the total energy content of body fat stores can exceed 10,000 kcal. In contrast, the body's total carbohydrate storage capacity is only 1% to 2% that of fat (approximately 1500-2500 kcal). This is due in part to the water required for storage, and the lower energy density (4 kcal/g) of carbohydrates. However, the nervous system and other tissues have an obligate requirement for carbohydrates. In addition, readily oxidized carbohydrates are necessary for peak physical performance since they support twice the rate of power production possible than when fat alone is combusted (6,7).

Some investigators have suggested that when food availability is limited, calorically dense, high-fat,low-carbohydrate combat rations are preferable since they minimize the drain on body fat stores and promote overall caloric balance (3). These rations, however, can result in reduced body carbohydrate stores, a transition to a fat-predominant fuel metabolism, and a decrement in the maximum sustainable endurance exercise intensity (6,8). On the other hand, consumption of low-fat, high-carbohydrate rations results in a more negative energy balance and increased use of body fat stores, but helps maintain body carbohydrate stores, provides the easily combusted fuel needed to sustain high rates of energy expenditure, and aids in conserving lean body mass (6,7,9,10).

The Committee on Military Nutrition Research of the National Research Council, Food and Nutrition Board, reviewed the concept of calorie-dense rations. They asserted that it is more important to provide at least 400 g carbohydrate/person/day to ensure at least partial muscle glycogen repletion than it is to maintain energy balance through a high fat ration (4). Evidence clearly shows that adequate dietary carbohydrate intake is necessary to maintain muscle

glycogen stores and provide fuel for sustained strenuous exercise (6,8). Maximum power production is greater, and the time needed to reach that level of power output is shorter, when carbohydrates are used as fuel (Table 1)(7,11). However, the typical carbohydrate intake of soldiers in the field consuming field rations averages only 300 g/day (12). This is probably inadequate to maintain body carbohydrate stores given that such stores are only about 500 g, and approximately 0.5 to 2.5 g of carbohydrate are oxidized per minute during exercise at 30% to 75% of maximum aerobic capacity ($\dot{V}O_2max$), respectively (8,13).

Table 1

Power production and time to maximum power production with fat and carbohydrate fuels

Aerobic Metabolism	Maximum Power[1]	Time to Reach Max. Power (min)
Carbohydrates -> $CO_2 + H_2O$	2.7	3.0
Free fatty acids -> $CO_2 + H_2O$	1.4	30.0

[1]Maximum power, expressed as mmol ATP/kg dry muscle/sec, was calculated assuming that 72% of a $\dot{V}O_2max$ of 4 L/min was utilized by a working muscle mass of 20 kg (4.7 kg dry mass). The maximum power of free fatty acid oxidation was assumed to correspond to the apparent upper limit of 50% of $\dot{V}O_2max$ (17)(adapted from 7).

The effects of dietary fat intake on fat and carbohydrate oxidation were studied over 9 to 24 hours in resting or slightly active individuals who were in neutral or positive energy balance (14-16). These studies demonstrated that the proportions and rates of fat and carbohydrate oxidation are not influenced by the fat content of the diet. However, little information is available on the physiologic effects of fat intake on physically active individuals in negative energy balance over dietary periods in excess of 24 hours.

STUDY OBJECTIVES

Eight male subjects were tested over two five-day periods. Each iteration consisted of four days of regular exercise, with approximately 4500 kcal/day total energy expenditure, followed by a test of endurance exercise capacity on day 5. During each trial, volunteers consumed either a basal hypocaloric diet (2300 kcal, 40% fat Calories), or the same diet with a 1000 kcal/day of additional fat (3300 kcal, 59% fat Calories). The broad objective was to assess whether the addition of fat calories to a basal diet had any influence on the fat balance, carbohydrate balance, fuel metabolism, or endurance exercise performance of physically active soldiers.

It was hypothesized that short-term lipid fuel requirements could be met equally well from either dietary sources or from body fat stores. Lowering total caloric intake by reducing the absolute amount of dietary lipid was not expected to result in decreased endurance exercise performance or undesirable physiologic consequences. It was also hypothesized that the metabolic demand for carbohydrates during the four-day exercise regimen would not be met by a dietary carbohydrate intake of 300 g/man/day.

The results of two ancillary studies, the "Acceptability of NSM demonstration rations," and the "Impact of a high-fat diet on the fecal microflora of adult male subjects" are presented and discussed in Appendices B and C.

METHODS

Eight male military volunteers with normal fasting blood cholesterol and triglyceride levels and no history of diabetes gave their informed consent to be studied under a protocol approved by the USARIEM and USAMRDC/OTSG Human Use Review Committees.

SUBJECT CHARACTERISTICS

At the start of the experiment, body composition was assessed by underwater weighing (18), and the subjects were familiarized with the exercise tests and equipment. A progressive continuous treadmill test to exhaustion was used to determine the maximum aerobic capacity ($\dot{V}O_2max$) of each subject (19). Subjects walked on a treadmill for 1 minute (3 mph, 1.34 ms^{-1})

and then ran at 6 mph (2.68 ms^{-1}) and 0% grade for 5 minutes. The grade was then increased to 5%, with additional 2.5% increases every 3 minutes until the subjects were exhausted. The $\dot{V}O_2max$ was remeasured after four weeks to determine whether any increase in aerobic capacity in response to physical training had occurred.

EXPERIMENT DESIGN

A counterbalanced, crossover design was used. There were two factors (basal diet, basal diet plus additional fat calories) with repeated measures (time). See Table 2 for the composition of the two diets. The eight subjects were randomly divided into two groups of four. The groups were studied alternately for five days at a time over a four-week period. The weekly test routine is outlined in Table 3. Physically active test weeks were alternated with sedentary weeks to minimize any training effects (20). The temperature in the exercise room was approximately 21°C.

Table 2
Composition of test rations

	Test Diets	
	Basal Diet	Basal Diet Plus Additional Fat Calories
Protein (g)	70.3 ± 1.9	69.8 ± 1.1
Carbohydrate (g)	285.1 ± 5.2	293.7 ± 2.7
Fat (g)	104.9 ± 3.5	207.1 ± 3.0
Fat (% kcal)	40	57
Energy (kcal)	2346 ± 29	3299 ± 15
Weight (g)	518 ± 1	621 ± 1

Table 3
Five-day test cycle

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Endurance exercise capacity						X
Resting gas exchange		X	X	X	X	X
Body weight	X	X	X	X	X	X
Blood substrates		X				X
Standardized intermittent exercise		X	X	X	X	
24-hr fecal collection					X	
24-hr urine collection					X	
Ration intake		X	X	X	X	
Ration acceptance		X	X	X	X	X
Treadmill endurance test						X

Two iterations of this test sequence (basal diet, basal diet plus additional fat Calories) were performed for each subject.

The exercise program from days 1 to 4 consisted of three 75-minute intermittent exercise sessions each day, two in the morning and one in the afternoon. The subjects rotated among four exercise machines: a treadmill, a cycle ergometer, a rowing ergometer, and a cross-country ski machine. Each session consisted of four 15-minute bouts on a given machine at a heart rate of 155±5 beats/minute, interspersed with 5–minute rest periods. During testing the subjects were restricted to an area consisting of a dormitory, kitchen, exercise room, and lavatory. Exercise was permitted only during exercise periods.

On day 5, endurance exercise capacity was determined by having the postabsorptive subjects wear a 15-kg military backpack during the continuous progressive treadmill test to exhaustion. Treadmill speed was kept constant at 6 kmh^{-1} (3.73 mph). The grade was level (0%) for the first 10 minutes, increased to 2% at minute 10, to 4% at minute 20, with additional 1% increases in grade every 10 minutes until the subject was exhausted. Heart rate, and

venous blood metabolite levels were measured. Gas exchange was determined with a calibrated metabolic cart (Sensor Medics, Anaheim, CA).

FOOD COMPOSITION AND CONSUMPTION

During days 1 to 4 of the test weeks the subjects consumed either a basal hypocaloric diet, or the same diet with an additional 1000 kcal/day of fat. The two diets, packaged as Prototype Nutritional Sustainment Module (NSM) rations, were similar in appearance (see Appendix A for a detailed description). Only the investigators directly involved in feeding the subjects were aware of who was receiving which diet. The added fat was spread over a number of different food components to produce a minimal difference in appearance between the two test diets. Both the size and types of food bars differed between the diets (see Appendix A). Carbohydrate and protein intakes were kept constant at approximately 300 g/day and 70 g/day, respectively. The rations were consumed in three scheduled meals plus one snack under the supervision of a data collector. Complete consumption of the food was encouraged. No food was available between meals. Water and caffeine- and calorie-free beverages were available ad libitum. A dietitian instructed each subject how to record fluid intake and hedonic ratings accurately during test weeks, and how to consume a balanced mess hall diet adequate in calories and carbohydrates during sedentary periods between test cycles.

The food items consumed by the subjects were weighed to the nearest gram. Nutrient composition data were provided by the Natick Research Development and Engineering Center. The test diets were analyzed for protein (total nitrogen) by the Kjeldahl method, and for fat by the acid hydrolysis method (21). Carbohydrates were estimated by subtracting the moisture, protein, fat, and ash percentage from 100%. Nutrient intakes were calculated from food consumption and food composition using a computerized nutrient analysis system developed at USARIEM (22).

RESTING GAS EXCHANGE

At 0600 to 0700 hours on days 1 to 5, the $\dot{V}E$, $\dot{V}CO_2$, and $\dot{V}O_2$ of resting, postabsorptive, supine subjects were determined immediately on awakening. Expired gas was collected in Douglas bags and analyzed by Perkin-Elmer respiratory mass spectrometer against known standard gases. The volume of expired gas was measured with a Tissot spirometer. The subjects were instructed to rest quietly without sleeping during gas collections. They breathed through a mouthpiece for 5 minutes before the start of two sequential 10-minute gas collection

periods. After gas exchange measurements, daily postabsorptive seminude body weights were measured with a calibrated digital scale accurate to ± 50 g (model E-1200; Sauter, Albstadt 1–Ebingen, West Germany).

ENERGY EXPENDITURE AND SUBSTRATE OXIDATION

During the exercise periods (days 1-4) subjects used an ambulatory heart rate monitor (Uniq Heart Watch, Computer Instruments Corp. Inc., Hempstead, NY) to maintain a target heart rate of 155 beats per minute. Since they found it difficult to work at different individual heart rates, no attempt was made to have identical energy expenditures. A submaximum treadmill test was used to determine the linear relationship of submaximum heart rate to $\dot{V}O_2$ for each subject. There were no significant differences in the individual heart rate-$\dot{V}O_2$ relationship among the various exercise machines. The total amount of oxygen consumed during the 240 minutes of intermittent exercise each day was estimated from the minute-by-minute heart rate records and the equations relating heart rate to $\dot{V}O_2$ (23).

The subjects were either sedentary or sleeping except during exercise periods. Oxygen consumption during sedentary periods was estimated by multiplying individual resting morning $\dot{V}O_2$ by the total number of sedentary waking hours. This value was then multiplied by a factor of 1.3 to correct for the increase in $\dot{V}O_2$ associated with minimum activity (24). Oxygen consumed during sleep was estimated by multiplying the resting $\dot{V}O_2$ by the total time spent sleeping and subtracting a 3% correction factor (15).

Mean total energy expenditure during days 1 to 4 was estimated from the total amount of oxygen consumed during rest, sleep, and exercise. Daily resting respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) measurements were used to estimate the caloric value for oxygen consumed during rest and sleep. The RER during intermittent exercise was interpolated from exercise RER measurements made before and on day 5 of the test weeks. Daily carbohydrate and fat balances were calculated from oxygen consumption and RER using standard calorimetric relationships (25,26) and dietary records. Daily substrate (carbohydrate, fat, and protein) balances were calculated as the difference between intake and oxidation (substrate balance = substrate consumed - substrate oxidized). The rate of protein oxidation, which was negligible as indicated by the nitrogen balance measurements, was assumed to be constant. The thermic effect of food was estimated as 14% and 10% of caloric intake for the basal diet without and with additional fat calories, respectively (15).

BLOOD ASSAYS

On the mornings of days 1 and 5, fasting venous blood samples were collected. Blood samples were collected in Na^+EDTA to determine metabolite levels, in Na^+EDTA/fluoride to measure lactate levels, in aprotinin (Trasylol)/Na^+EDTA/glutathione to determine hormone levels, or allowed to clot and the serum collected to perform automated analyses. Samples were stored at -80°C. Hemoglobin and hematocrit were determined on well-mixed, anticoagulated whole blood (model S880; Coulter Corp., Hialeah, FL). Free fatty acid, glycerol, ketone, glucose, and lactate levels were measured with enzymatic or colorimetric assays. Insulin and cortisol were measured by radioimmunoassay. Glucose, blood urea nitrogen, creatinine, cholesterol, triglycerides, high-density lipoprotein cholesterol, total protein, albumin, sodium, potassium, chloride, and iron levels were determined by automated analyzer (SmithKlein Bio-Science Laboratories, Waltham, MA).

FECAL TRANSIT TIME AND FECAL FAT LEVEL

Fecal transit times were determined by having the subjects ingest 1.0 g nontoxic, nondigestible, nonabsorbable carmine red dye (Sigma Chem. Co., St. Louis, MO) with their first meal, and noting the time of appearance of red feces (27). Fat and nitrogen levels were measured in 24-hour fecal samples collected the day after red feces appeared. Feces were collected, homogenized, and weighed, and a 20-g sample was frozen for subsequent quantitative analysis for fat by the gravimetric method (SmithKlein Bio-Science Laboratories, Waltham, MA). A separate 1-g sample was analyzed for microflora (see Appendix C).

NITROGEN BALANCE

Twenty-four hour urine (N=8) and fecal samples (N=4) were collected for urea and total nitrogen measurements on either day 3 or 4 of each study period depending on when the rations were being excreted as signaled by passage of red dye. Fecal and acidified urine samples were immediately refrigerated. One subject was excluded from the nitrogen balance study because he did not consume all of the ration on the day of the test. Urinary nitrogen was by the Kjeldahl procedure. Nitrogen intake was calculated as grams of dietary protein intake divided by 6.25. Nitrogen output was calculated as the sum of urinary, stool, skin, and sweat losses. Skin and sweat nitrogen losses were estimated to be 0.25 g/m^2 body surface area/day (28). Nitrogen balance was equal to nitrogen intake minus nitrogen output.

STATISTICAL ANALYSIS

The data were analyzed by a two-way (diet, time) analysis of variance with repeated measures with a post hoc analysis using the least significant difference test for between-diet comparisons (29). Differences were considered significant at the $P<0.05$ level. All results are reported as means ± SEM.

RESULTS

SUBJECT CHARACTERISTICS

The initial physical characteristics of the subjects are shown in Table 4. The $\dot{V}O_2max$ did not change over the course of the experiment (4.08±0.22 $L \cdot min^{-1}$ preexperiment; 4.11±0.22 $L \cdot min^{-1}$ postexperiment)($P>0.05$). This indicates that the confounding effects of changes in physical conditioning were avoided by alternating physically active test weeks with sedentary rest weeks.

Table 4
Physical characteristics of subjects

Variable	Mean ± SEM	Range
Age (yrs)	22.00 ± 1.30	18.00 - 30.00
Height (cm)	181.30 ± 1.50	175.30 - 188.00
Weight (kg)	80.70 ± 4.50	66.40 - 101.40
Body fat (%)	17.90 ± 2.60	9.00 - 30.00
$\dot{V}O_2max$ (L/min)	4.08 ± 0.22	3.29 - 5.25
$\dot{V}O_2max$ (ml/kg/min)	51.00 ± 2.30	44.10 - 57.50

FOOD AND WATER CONSUMPTION

Since the subjects consumed all of the food they were given, daily energy, fat, protein, and carbohydrate intakes were equivalent to the compositions of the diets. As anticipated, energy and fat consumption differed widely between diets, while dietary protein and carbohydrate intakes were similar. Fluid intake was similar for the diets (Table 5).

Table 5
Dietary water intake

	Basal Diet	Basal Diet Plus Additional Fat Calories
Water drunk (ml)	6184 ± 426	5799 ± 481
Water from food moisture (ml)	32 ± 1	32 ± 1
Water added to food (ml)	267 ± 22	379 ± 21
Total water intake (ml)	6484 ± 424	6209 ± 479

ENERGY EXPENDITURE AND BODY WEIGHT CHANGES

No significant difference was recorded between the calculated energy expenditures of the subjects eating the basal diet (4578±215 kcal/d) and the diet with additional fat calories (4566±231 kcal/day). Since the target heart rate during exercise was 155±5 beats/minute for all subjects regardless of $\dot{V}O_2$max, the three largest subjects with higher absolute $\dot{V}O_2$max had slightly higher rates of energy expenditure. Nevertheless, exercise intensity, which was approximately 60% of $\dot{V}O_2$max, did not differ between the diets.

During the four days preceding the endurance tests body mass decreased significantly, from 81.1±3.8 kg to 78.7±3.6 kg with the basal diet and from 81.0±4.2 kg to 79.3±4.0 kg with the diet with additional fat calories ($P<0.05$)(Figure 1). The mean values suggest a tendency for a less rapid loss of body mass on the diet with added fat calories. However, no significant differences in body weight loss were noted between the diets ($P>0.05$)(Appendix D), presumably because of the relatively short duration of the test.

GAS EXCHANGE, BLOOD SUBSTRATES, AND SUBSTRATE BALANCES

Dietary effects at rest

The 1000 kcal/d of additional dietary fat had no effect on resting, postabsorptive, basal levels of plasma free fatty acids (FFA), β-hydroxybutyrate (BOHB), glycerol, triglycerides (TG), glucose, lactate, insulin, or cortisol (Figures 2-9). A tendency toward lower FFA and higher BOHB levels was seen with the basal diet, but the differences were not statistically significant. All basal postabsorptive blood chemistries (blood urea nitrogen, creatinine, total cholesterol, HDL cholesterol, total protein, albumin, sodium, potassium, chloride, and iron) were within normal limits, with no effect of diet on blood chemistries with the minor exception of a small increase in blood albumin with the diet with additional fat calories (Table 6). There was also no effect of dietary lipid level on daily basal postabsorptive resting RER (Figure 10).

Time effects at rest

In contrast to the limited effects of diet, physiologic changes were significant through time in each exercise period. Resting RER decreased significantly from 0.91±0.02 on day 1 to 0.82±0.01 on day 3, and then remained at approximately 0.82 through the morning of day 5. Resting plasma TG, glucose, lactate, and insulin levels also decreased significantly, whereas plasma levels of FFA and BOHB increased significantly. Resting plasma glycerol and cortisol levels were unchanged.

Diet effects on substrate oxidation and endurance exercise capacity

The addition of fat to the basal diet had no effect on heart rate (Figure 11), oxygen consumption (Figure 12), substrate oxidation as indicated by RER (Figure 13), blood substrate and hormone levels, or endurance capacity of the subjects during the endurance exercise test. Time to exhaustion during the endurance test was the same with (106.5±7.6 min) and without (106.1±11.2 min) additional fat calories. Subject No. 3 stopped exercise prematurely due to a viral infection during the basal diet test cycle (Appendix D). When this individual was excluded from the calculations of endurance capacity, the endurance times change slightly (basal diet,

113.81±9.30 min; basal diet plus additional fat calories, 107.91±8.71 min). However, there continued to be no statistical effect of additional dietary fat on endurance capacity.

Time effects during exercise

Heart rate and oxygen consumption increased progressively during the endurance exercise test, reaching a maximum of 174±12 and 177±8 beats/minute and 2.84±0.23 and 2.91±0.21 L/min with the basal diet and the basal diet plus additional fat calories, respectively. The RER at the start of the endurance exercise test averaged 0.73±0.01 for both regimen, significantly lower than the day 5 resting value. This decrease in RER from rest to exercise is probably due to the fat-predominant fuel oxidation of glycogen-depleted muscles during exercise. At the end of the bout of endurance exercise, RER had increased significantly ($P<0.05$) to 0.82±0.02 with the basal diet and to 0.81±0.02 on basal diet with added fat calories. Nevertheless, fat remained the predominant fuel oxidized. During endurance exercise, plasma TG, glycerol, and lactate increased significantly ($P<0.05$), but there were no significant changes in FFA, BOHB, glucose, insulin, or cortisol levels ($P>0.05$).

Overall energy and substrate balances

Energy, fat, and carbohydrate balances are shown in Figure 14. Overall, the addition of fat calories improved fat balance but had no effect on carbohydrate balance. Daily energy balance, which did not vary significantly during the four days of intermittent exercise, averaged −2277±215 kcal/day with the basal diet and -1261±232 kcal/day when additional fat calories were consumed. Additional dietary fat resulted in a significantly more positive fat balance (basal −1191±218 kcal; additional fat -227±181 kcal)($P<0.05$). The mean difference in energy balance between the diets (872±51 kcal/day) was equal to 89±5% of the kcal of the 981-kcal/day of additional dietary fat. The addition of dietary fat had no effect ($P>0.05$) on the transition in time to a more fat-predominant fuel metabolism and a less negative carbohydrate balance. In addition, it had no effect ($P>0.05$) on the four-day mean carbohydrate balance (basal −1090±140 kcal/day; additional fat −1031±220 kcal/day) or on nitrogen balance (basal −5.5±0.9 g/day; additional fat −3.7±1.3 g/day).

FECAL TRANSIT TIME AND FECAL FAT LEVEL

No statistical difference ($P>0.05$) was seen in intestinal transit times between diets (basal 24.3±7.3 hrs; additional fat 14.7±4.1 hrs). Transit times varied widely. This variability was due in part to the transient viral gastroenteritis experienced by a subject who ate the basal diet, and the constipation experienced by one who ate the diet with additional fat. Subjects who ate the diet with additional fat excreted more fat in their feces (basal 4.2±1.3 g/day; additional fat 9.3±1.7 g/day)($P<0.05$). However, fat excretion expressed as a percentage of fat intake was similar without (4.0%) and with additional fat calories (4.5%), indicating that fat absorption was normal and similar for both diets. This additional fecal fat accounted for approximately half of the difference between the 981 kcal/day of additional dietary fat and the calculated 872 kcal/day difference in fat balance between the diets.

Table 6
Blood chemistry

	Basal Diet		Diet With Additional Fat		Normal Adult Range[1]
	Pre	Post	Pre	Post	
Blood urea nitrogen (mg/dl)	13.4 ± 1.2	13.3 ± 0.6	14.3 ± 1.2	14.5 ± 1.3	7.0 - 25.0
Creatinine (mg/dl)	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.7 - 1.4
Total cholesterol (mg/dl)	172.1 ± 9.9	154.0 ± 6.5	175.1 ± 0.6	167.4 ± 5.3	170.0 - 260.0
HDL cholesterol (mg/dl)	49.3 ± 5.4	52.3 ± 7.3	44.3 ± 5.5	54.3 ± 10.1	28.0 - 62.0
Total Protein (g/dl)	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.7 ± 0.1	6.0 - 8.5
Albumin (g/dl)	4.6 ± 0.1	4.3 ± 0.1[2]	4.3 ± 0.1	4.6 ± 0.1[2]	3.2 - 5.5
Sodium (mEq/L)	140.6 ± 0.5	139.9 ± 0.9	140.5 ± 0.9	142.3 ± 0.9	135.0 - 148.0
Potassium (mEq/L)	4.4 ± 0.1	4.6 ± 0.1[3]	4.4 ± 0.2	4.4 ± 0.1	3.5 - 5.3
Chloride (mEq/L)	105.1 ± 1.1	105.5 ± 0.7	105.3 ± 0.7	106.6 ± 0.7	95.0 - 110.0
Iron (mg/dl)	102.0 ± 12.6	130.3 ± 8.8[3]	105.0 ± 21.8	137.0 ± 11.3[3]	40.0 - 175.0

Blood chemistries represent $\bar{X}$ ± SEM, N = 8.

[1] Siest, G., et al., editors: Interpretation of Clinical Laboratory Tests, Biomedical Publications, Foster City, California, 1985.

[2] Significant difference between ration groups ($P<0.05$).

[3] Significant difference from preexperiment to postexperiment within ration group ($P<0.05$).

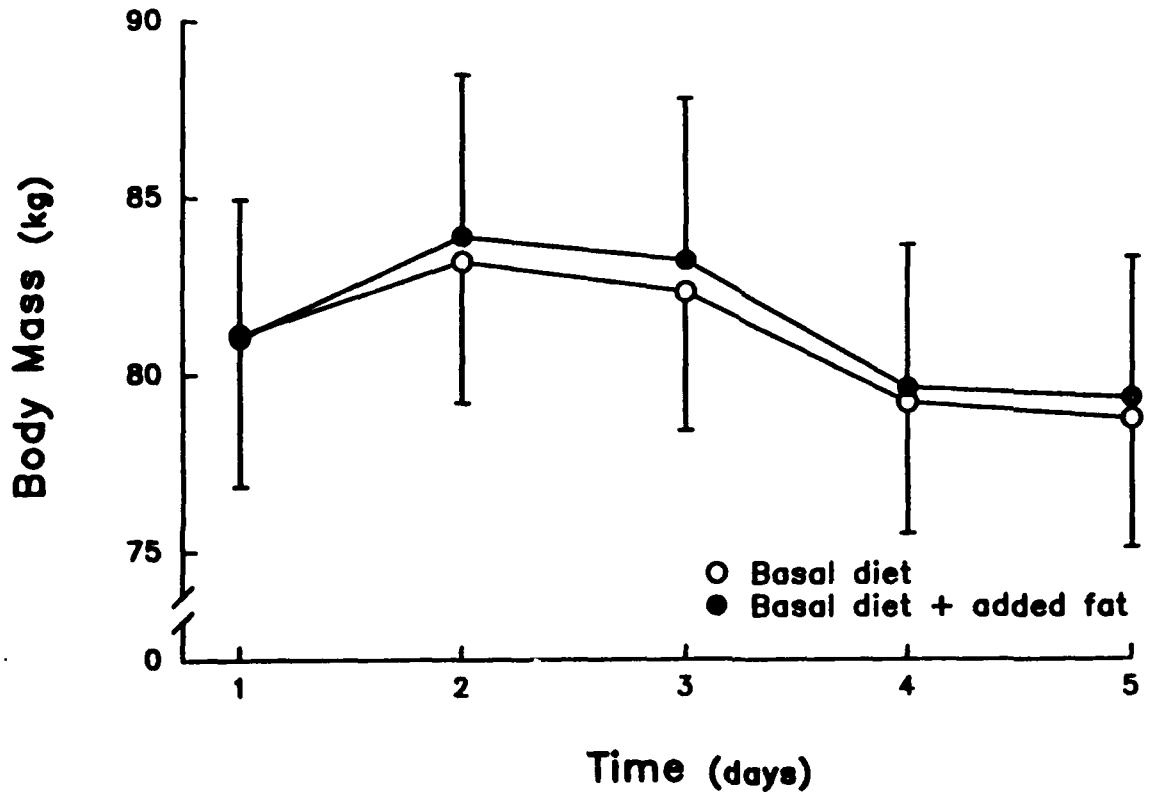

Figure 1. Change in postabsorptive morning body mass during the five day test cycle. Body mass decreased significantly from day 1 to day 5 ($P<0.05$). There was no significant effect of added dietary fat on the loss of body mass ($P>0.05$).

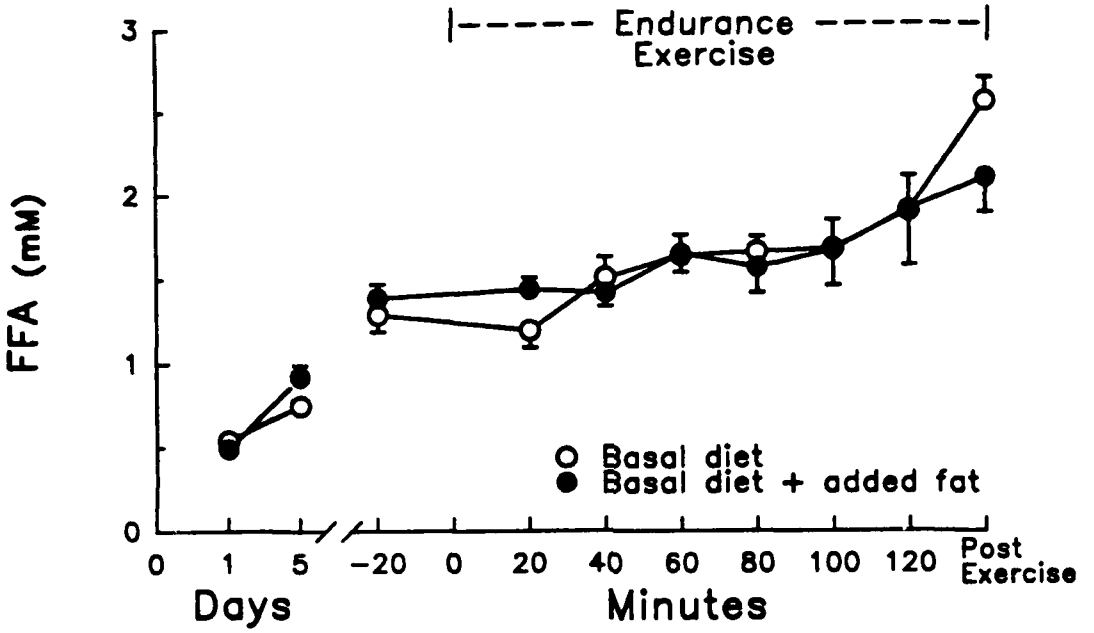

Figure 2. Resting plasma free fatty acid (FFA) levels increased significantly from day 1 to day 5 ($P<0.05$). There was no significant change in plasma FFA concentration during endurance exercise ($P>0.05$). Added dietary fat had no effect on plasma FFA concentrations at rest or during endurance exercise ($P>0.05$).

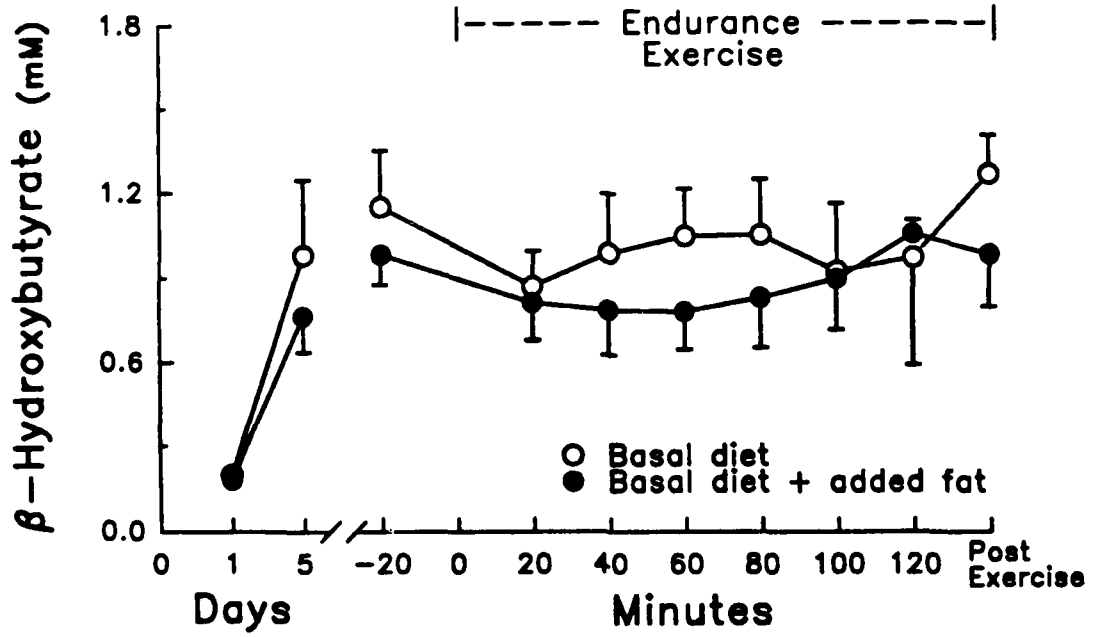

Figure 3. Resting plasma levels of β−hydroxybutyrate increased significantly from day 1 to day 5 ($P<0.05$), but were unchanged during endurance exercise ($P>0.05$). Additional dietary fat had no effect on plasma β−hydroxy−butyrate concentrations at rest or during endurance exercise ($P>0.05$).

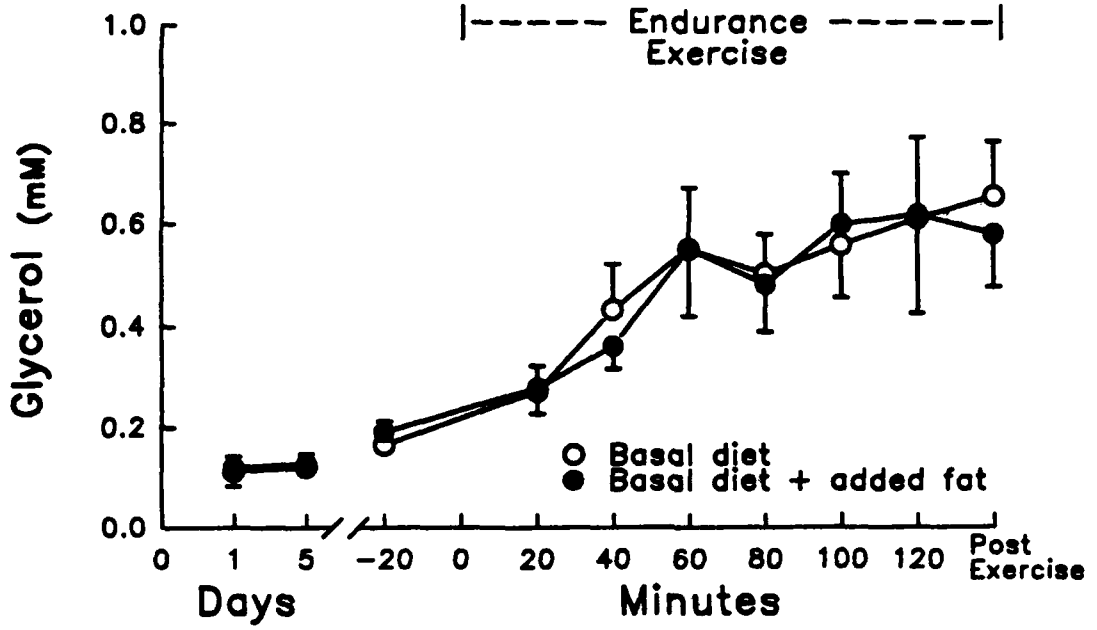

Figure 4. Resting plasma glycerol levels were unchanged from day 1 to day 5 ($P>0.05$), but increased during endurance exercise ($P<0.05$). Additional fat intake had no effect on plasma glycerol concentrations at rest or during endurance exercise ($P>0.05$).

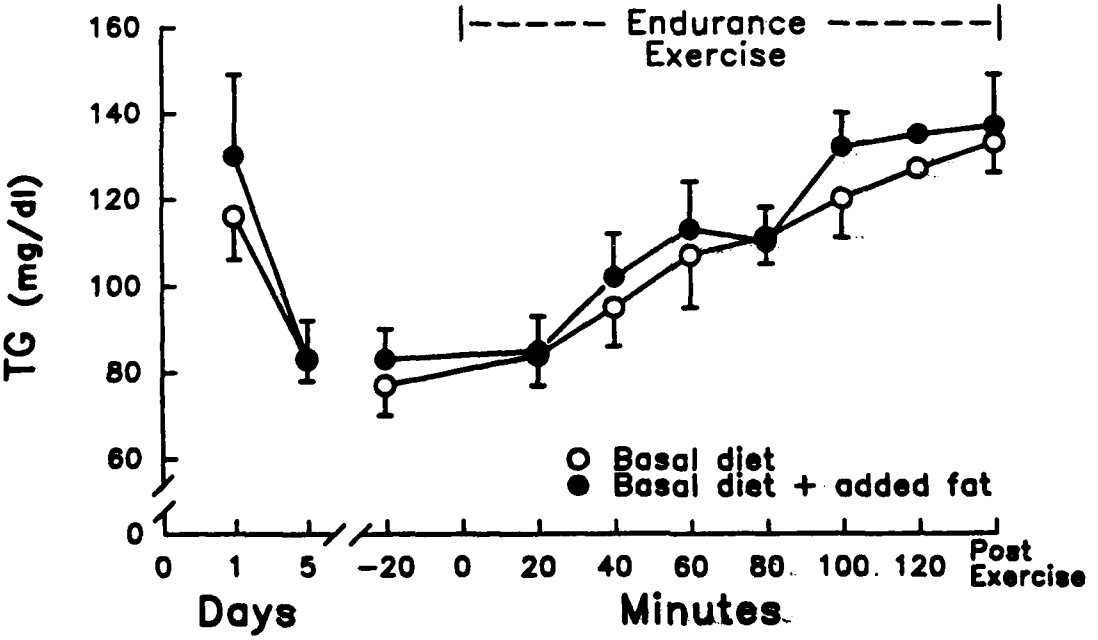

Figure 5. Resting plasma triglyceride concentrations decreased significantly from day 1 to day 5 ($P<0.05$), and then increased significantly during endurance exercise ($P<0.05$). Additional dietary fat had no effect on plasma triglyceride concentrations at rest or during endurance exercise ($P>0.05$).

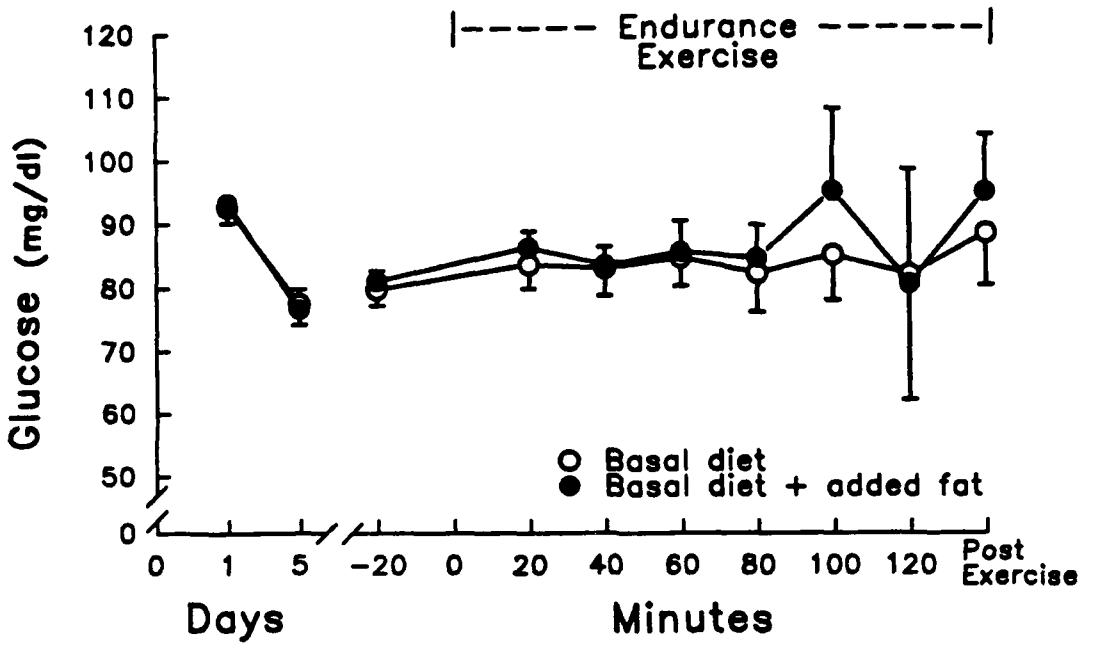

Figure 6. Resting plasma glucose concentrations decreased significantly from day 1 to day 5 ($P<0.05$), but did not change significantly during endurance exercise ($P<0.05$). Additional fat intake had no effect on plasma glucose concentrations at rest or during endurance exercise ($P>0.05$).

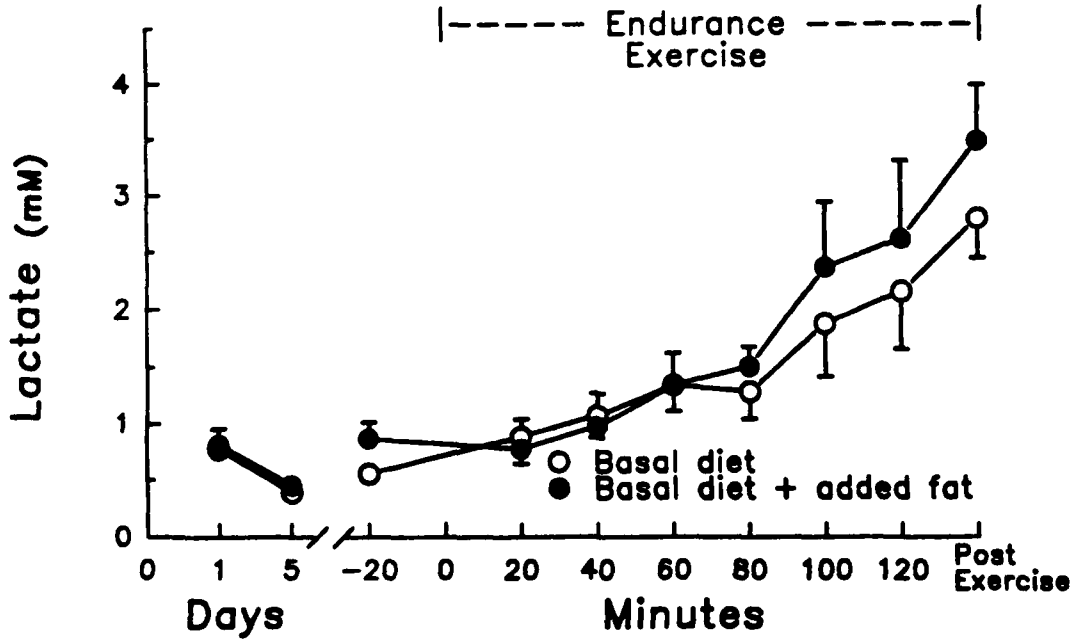

Figure 7. Resting plasma lactate levels decreased significantly from day 1 to day 5 ($P<0.05$), but increased significantly during endurance exercise ($P<0.05$). Additional dietary fat had no effect on plasma lactate concentrations at rest or during endurance exercise ($P>0.05$).

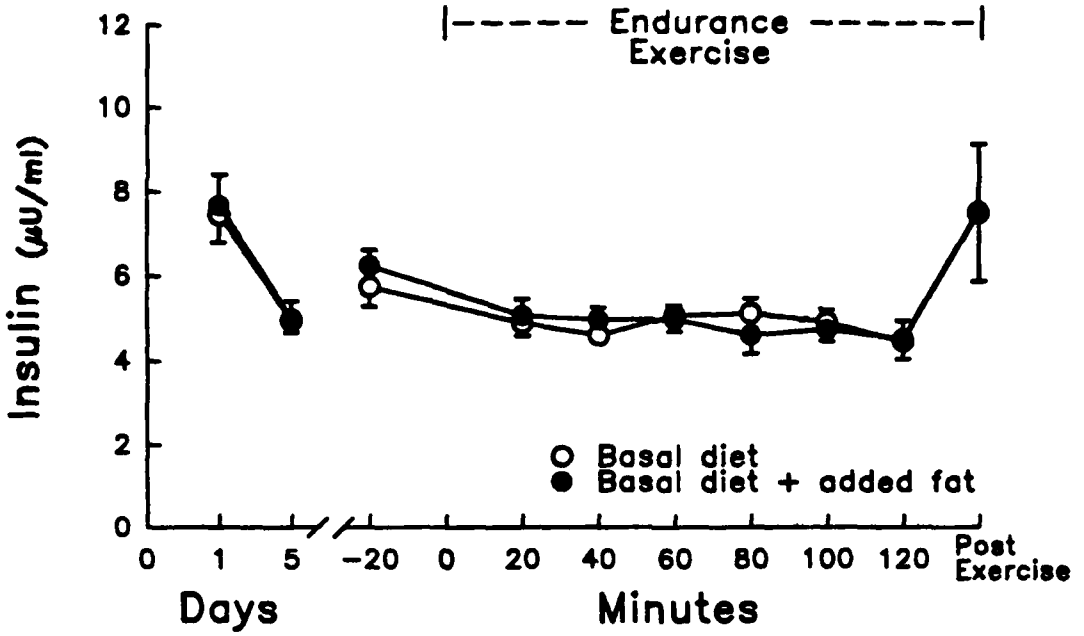

Figure 8. Resting plasma immunoreactive insulin levels decreased significantly from day 1 to day 5 ($P<0.05$), but were unchanged during endurance exercise ($P<0.05$). Additional dietary fat had no effect on plasma immunoreactive insulin concentrations at rest or during endurance exercise ($P>0.05$).

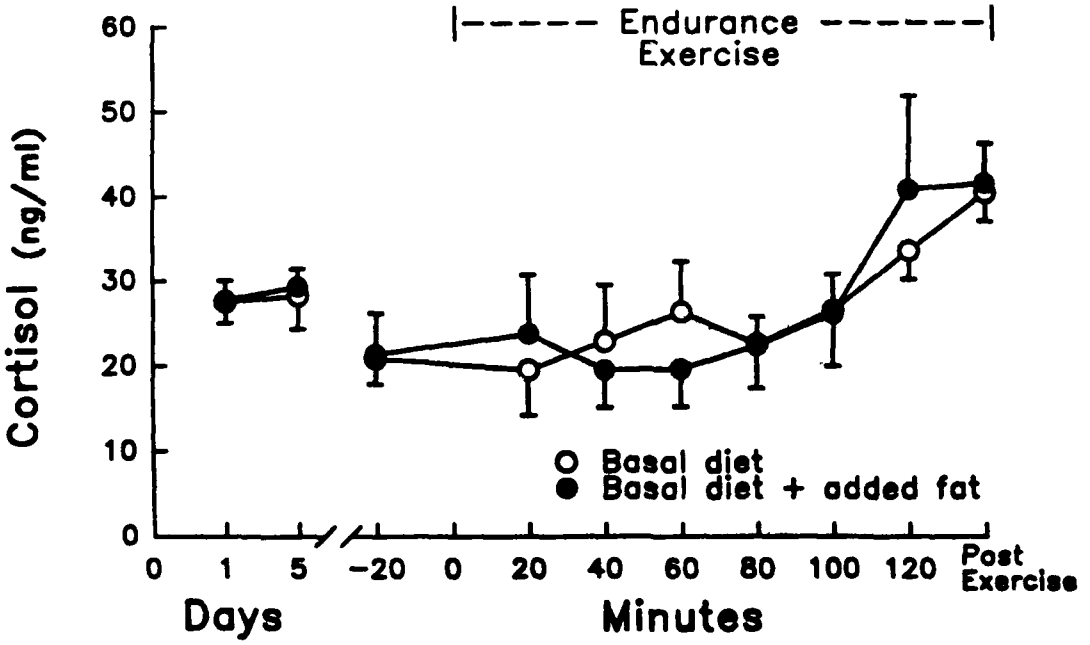

Figure 9. There were no significant changes in plasma cortisol levels at rest or during endurance exercise ($P>0.05$). Additional dietary fat had no effect on plasma cortisol concentrations at rest or during endurance exercise ($P>0.05$).

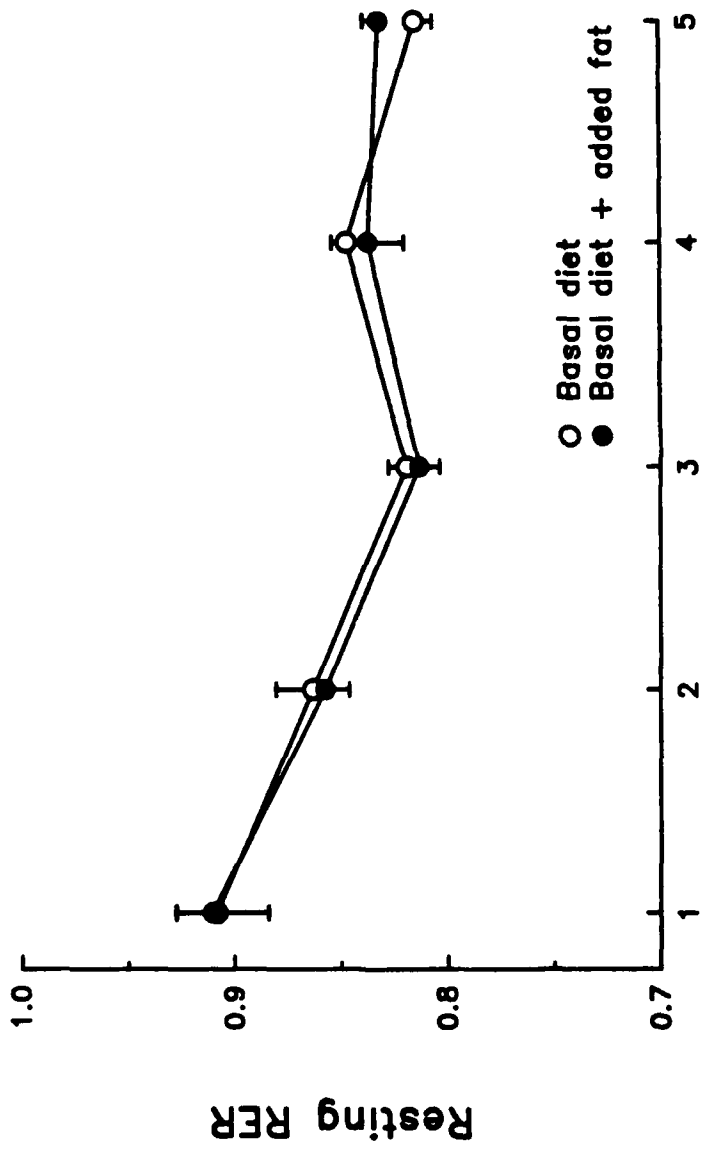

Figure 10. Resting respiratory exchange ratio (RER) decreased significantly by day 3 ($P<0.05$) and remained depressed through day 5. Additional dietary fat had no effect on this value ($P>0.05$).

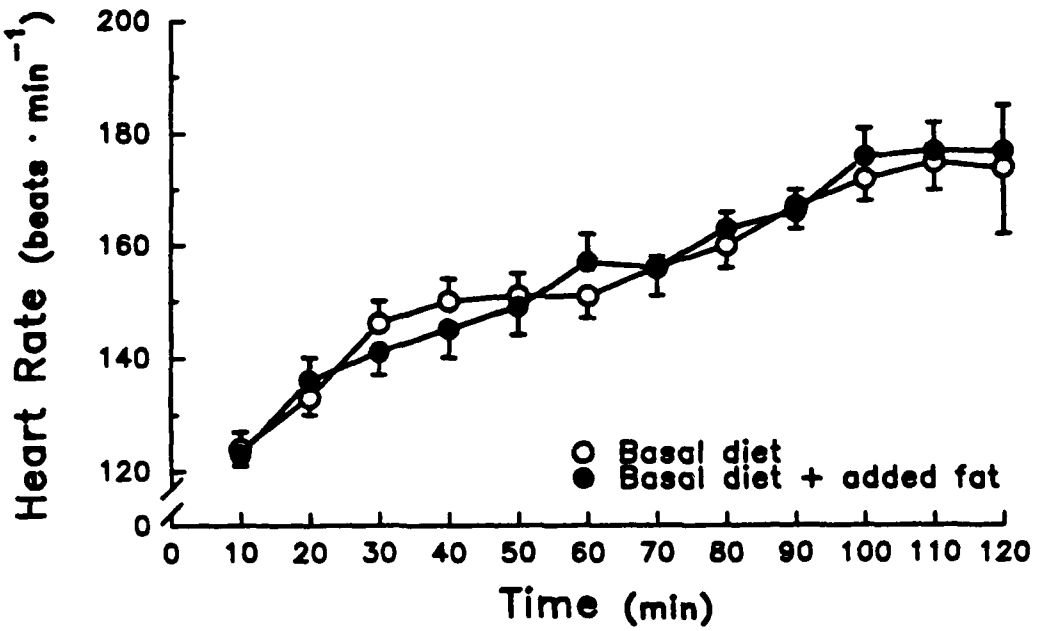

Figure 11. Heart rate during treadmill endurance exercise to exhaustion. Additional dietary fat had no effect on heart rate during endurance exercise (P>0.05).

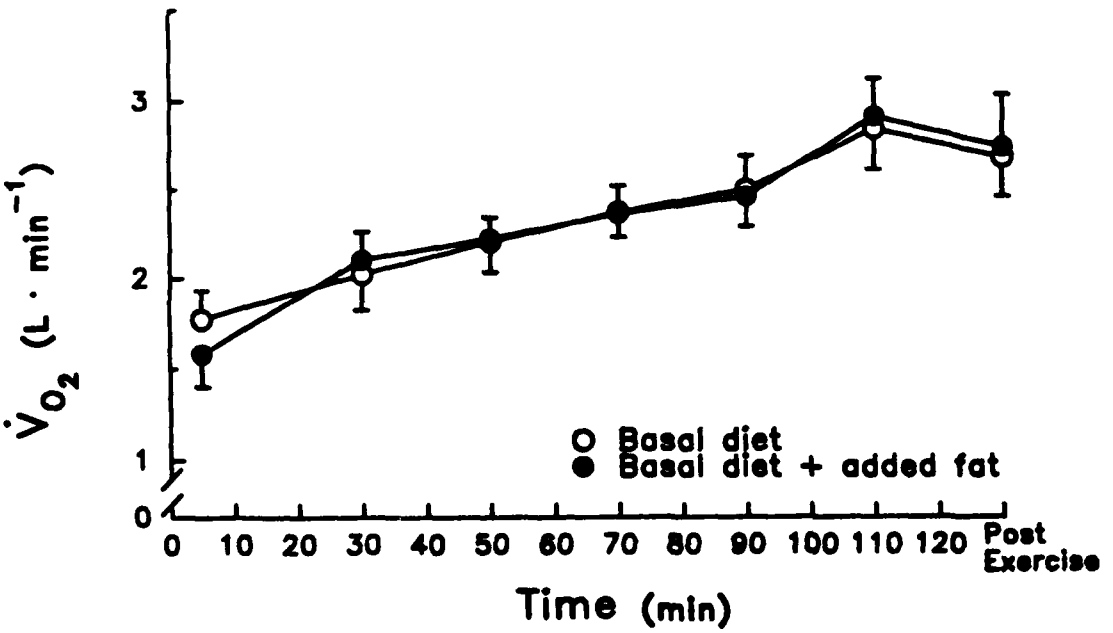

Figure 12. Oxygen consumption during treadmill endurance exercise to exhaustion. Additional fat intake had no effect on oxygen consumption during endurance exercise (P>0.05).

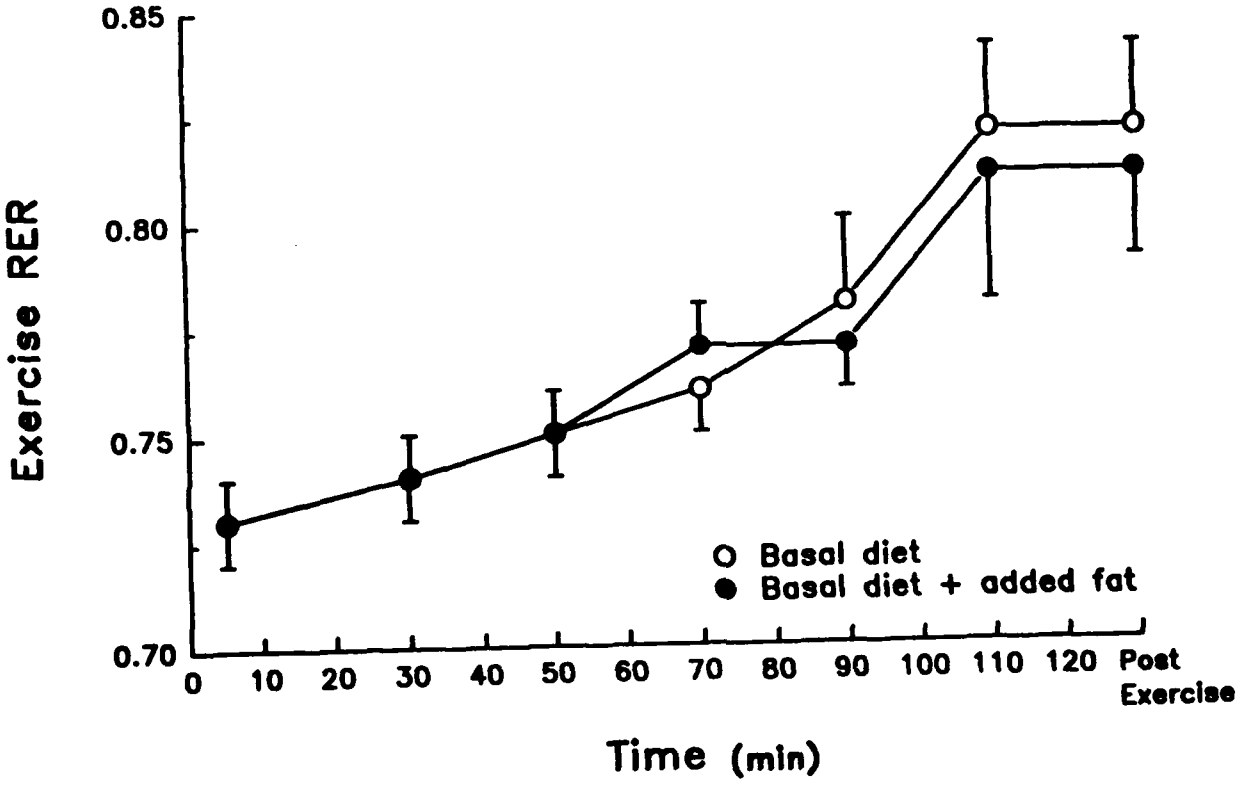

Figure 13. Respiratory exchange ratio (RER) increased significantly ($P<0.05$) during endurance exercise. However, additional dietary fat had no effect on oxygen consumption during exercise ($P>0.05$).

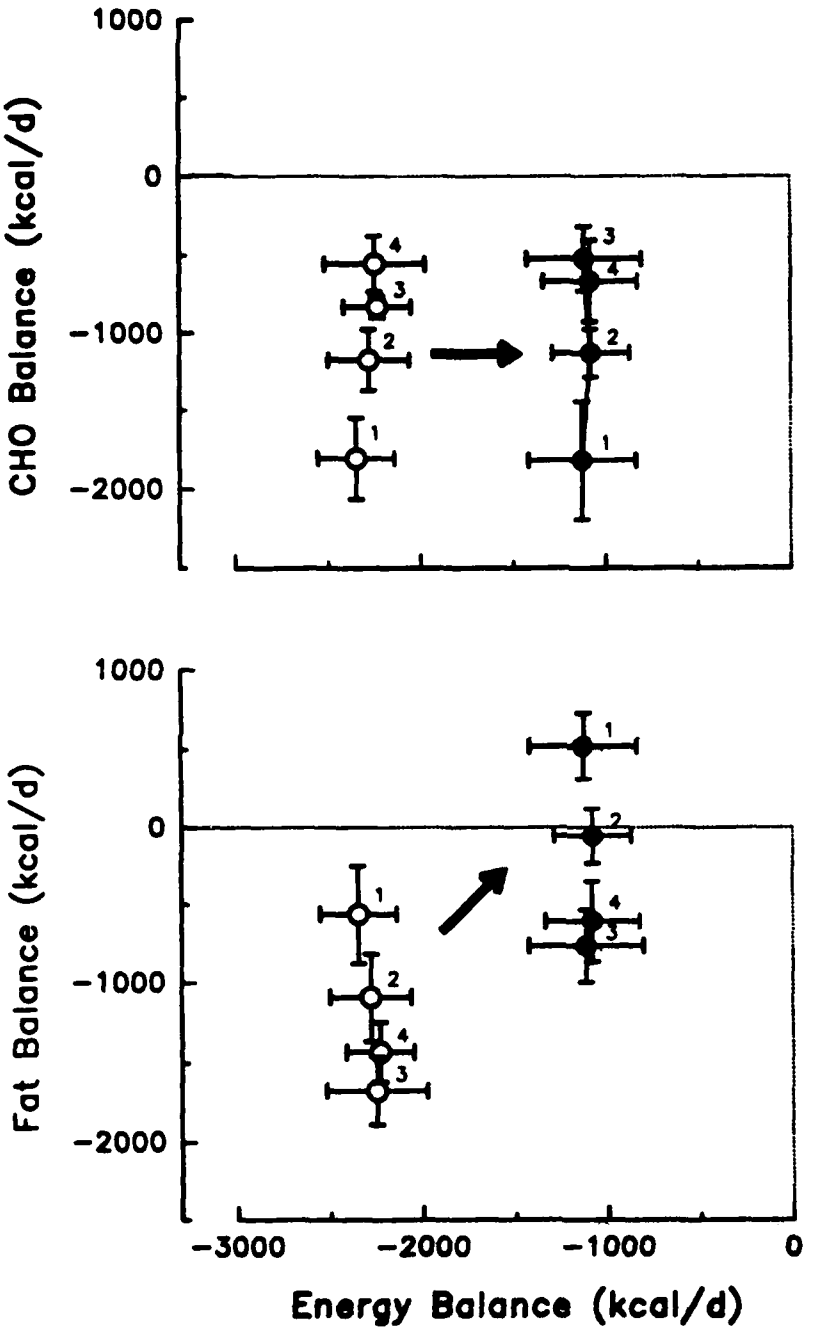

Figure 14. Relationship between carbohydrate balance, fat balance, and energy balance in subjects consuming either a calorie deficient basal diet (open circles) or the same diet plus a 1000 kcal/day of additional fat (filled circles). The numbers identify test days 1 through 4 during each test cycle. Values on each test day are means for all eight subjects ± standard errors.

DISCUSSION

Many studies have compared the physiologic effects of diets with different macronutrient compositions but the same caloric content. Unfortunately, when diets with equal caloric contents are studied, the specific physiologic effects of changing fat intake cannot be determined since any change in dietary fat is associated with reciprocal changes in carbohydrate and/or protein intake. We avoided this by comparing the physiologic responses to a basal diet with and without additional dietary fat.

In this study, increasing dietary fat calories had no significant effect on carbohydrate balance, nitrogen balance, water intake, or endurance exercise capacity. These results are consistent with previous reports that increased fat intake had no significant nitrogen-sparing or carbohydrate-sparing effects (15,30). Healthy humans absorb up to 150-200 g/day, or approximately 94%, of total dietary fat consumed, and excrete up to 7 g/day on a typical diet (31,32). The fecal fat levels of the subjects were higher with the added dietary fat, approaching the upper clinical limit of normal. However, reports of modest increases in fecal energy content with additional fat intake suggest this is not unusual (16). Few physiologic effects of dietary fat intake were evident, even though the subjects were physically active and had negative energy and carbohydrate balances.

Substrate balance calculations provide further evidence that the level of dietary fat intake had little direct or immediate physiologic impact (see Figure 14). Fat balance (i.e., difference between fat intake and calculated fat oxidation) became more positive in direct proportion to the metabolizable energy content of the additional dietary fat. In addition, a more positive calculated fat balance, together with a modest increase in fecal fat excretion, accounted for about 95% of the energy content of the additional dietary fat. These results are consistent with reports that the addition of fat (long chain triglycerides) to the diet does not increase fat oxidation, and that 90% of the metabolizable energy of added dietary fat is retained as body fat (4,14-16). The National Research Council Committee on Military Nutrition concluded, "There appears to be no nutritional advantage for the short-term use of a high-fat diet by troops during combat operations" (4).

In a study of eight physically active young men fed a control diet for one week, and then fed the same diet with additional fat calories the next week, energy balance was positive and about 90% of the metabolizable energy of the additional dietary fat was deposited in body fat stores

(16). Flatt et al. (14) reported that increasing long-chain triglyceride intake did not influence the rates of fat and carbohydrate oxidation during nine hours after eating. In a respirometer chamber study, seven young men engaged in light, intermittent exercise and ingested a basal diet on one day and the same diet with additional fat calories during the next 36 hours (15). It was evident that the additional dietary fat was simply deposited in body fat depots and had no effect on fuel oxidation (15).

The effects of dietary fat content on ration acceptability or fecal microflora were also studied. The results of these ancillary studies, presented and discussed in Appendices B and C, also suggest that the fat content of the diet had no effect on either food acceptability ratings or intestinal microflora.

In contrast to the minimal physiologic impact of adding fat to the diet, significant biochemical and physiologic changes were seen from day 1 to day 5 that were attributable to the limited 300 g/day carbohydrate intake. When carbohydrate intake does not meet demand, for example during prolonged exercise or semistarvation, carbohydrate reserves are reduced, carbohydrate use decreases, and fat use increases. The decreases in resting respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$), and in plasma glucose, lactate, TG, and insulin levels during the test cycles, and the increases in plasma FFA and BOHB levels indicate a shift from a carbohydrate- to a fat–predominant fuel metabolism (33). The time course of this transition varies with the individual's diet, aerobic fitness ($\dot{V}O_2max$), duration and type of exercise, and environmental conditions (8,13,33-35). A transition to a fat-predominant fuel metabolism conserves carbohydrates for glucose–dependent tissues such as the central nervous system and red blood cells. Inadequate dietary carbohydrate intake can lead to reduced physical endurance at exercise intensities as low as 45% of maximum aerobic capacity (36). Prolonged consumption of a hypocaloric, low carbohydrate diet can also result in reduced muscle strength and even decreased morale (9). The Committee on Military Nutrition and Nutrition Standards for Operational Rations suggests that a carbohydrate intake of at least 400 to 450 g/day is necessary to avoid carbohydrate deprivation in physically active soldiers (4,37).

Resting RER decreased during the four-day diet periods from about 0.92 to 0.83, indicating that the contribution of fat to the resting fuel mix had increased from approximately 27% to 57%. However, daily postabsorptive, morning basal RER was not influenced by the added fat. Although the initial mean resting RER of 0.92 was higher than the 0.85 typically reported for resting adult men, we had no technical reasons to doubt the measurements. The relatively elevated resting values at the start of the dietary periods may have been related to sedentary

lifestyle and high-carbohydrate diet during the week preceding testing. Fuel oxidation was fat-predominant, even at elevated workloads when a more carbohydrate-predominant fuel metabolism would be expected, during the endurance exercise test on day 5. The contribution of fat to fuel metabolism was calculated from RER to be 90% at the start of exercise, 77% after 90 minutes of exercise, and 62% after 120 minutes of exercise. The end-exercise contribution of fat to fuel oxidation is probably an underestimate due to hyperventilation-induced increases in $\dot{V}CO_2$.

PRACTICAL RAMIFICATIONS

Apparently no immediate physiologic benefits are gained with high dietary fat intake since body fat stores are normally readily available to buffer any shortfall in intake. The typical soldier has substantial reserves to meet fat energy needs. For example, a typical young male soldier weighing 74 kg (163 lbs) has approximately 13.5 kg (29.6 lbs) of body fat (18). Approximately two-thirds, or 9 kg (19.8 lbs), of this can be drawn upon without encroaching upon nerve sheath lipids or other fats necessary for normal physiologic function. This energy reserve is equivalent to 69,300 kcal of energy assuming a body fat energy density of 7700 kcal/kg (3500 kcal/lb).

In this study, the 450 g basal diet and the 561 g diet with additional fat calories provided equivalent short term nutritional support. Soldiers in the field typically lose body weight when served field rations, drawing upon body fat reserves to compensate for differences between caloric intake and expenditure (reviewed in ref. 12). This study of short term use of very compact rations under a moderately stressful regime compared a typical field caloric intake of 2400 kcal with about 100 g fat and 40% fat Calories (the typical American diet is about 37% fat Calories) with the same diet with 100 g of extra fat to increase fat to a high 57% of Calories. No significant harmful effects on physical performance or alterations in biochemical indicators of metabolic status resulted from the addition of fat calories. However, because the additional dietary fat had no positive effect on physical performance, a trade-off analysis of the effects of replacing the 100 g fat with 100 g of carbohydrate and thereby increasing carbohydrate content is useful. For example, the addition of a 100 g carbohydrate supplement, rather than a 100 g fat supplement, to the basic ration we studied would yield a 2700 kcal ration that weighed the same as the 3300 kcal ration with added fat but would contain approximately 400 g carbohydrate/day. A minimum intake of 400 g/day is required to avoid carbohydrate depletion and decreased physical performance, and possibly decreased mental performance, during field exercises (4).

Soldiers engaged in physically demanding short-term field operations usually are in negative energy balance, with dietary energy intake that averages 2500 kcal/day and mean dietary carbohydrate intake of about 300 g/day (12). Although most soldiers have large body fat reserves and are not in danger of starvation, they risk carbohydrate depletion and decreased physical and mental performance (4,9,38). For the short term use intended for most field rations, a high carbohydrate diet, although lower in total Calories, would be an effective way to prevent or slow depletion of the body's limited carbohydrate (glycogen) reserves, and thereby maintain or restore the original ratio of carbohydrate-to-fat fuel metabolized by the soldier. Only when energy deficits are prolonged to the point that body fat reserves are depleted would a higher fat diet have an advantage over the carbohydrate supplemented diet because weight and volume constraints would allow a greater total caloric intake from the higher fat ration.

From a physiologic perspective, one of the most important and widespread uses of energy-dense lipids is for endogenous fuel storage (39). It is self-evident that during extended periods of energy deprivation soldiers with adequate energy stores have a competitive advantage over those with inadequate stores. Therefore, when field operations are prolonged beyond two to four weeks, it becomes increasingly important to minimize the drain on body fat stores or to avoid soldiers entering periods of severe energy imbalance with marginal reserves. High levels of food energy consumption in the field (4500 kcal/person/day) have been achieved only when hot, palatable food was available during regularly scheduled mealtimes (40). This indicates that improvements in the overall energy balance of soldiers are best attempted by providing, at the earliest possible time, hot, palatable A-rations rather than by increasing the fat content of packaged field rations.

This study demonstrates that during short term periods (less than 5 days), the caloric content of the ration is of lesser concern that the carbohydrate content. Whereas during extended periods of field ration use, when there is a risk of infringing upon nerve sheath lipids or other fats necessary for normal physiologic function, the total caloric content of the ration assumes greater significance. Hence, it may be unnecessary to acheive maximum caloric density in rations designed for short term combat use. However, it may be desirable to increase the caloric density of rations intended for extended periods of utilization. Both of these objectives could be acheived by developing two complimentary modules: the first module would meet the recommendations that packaged rations provide 400 to 440 g of carbohydrate and 70 g of protein per day and as much fat as practical within weight and volume constraints. The second module would consist of calorically dense foods that complimented those found in the first module and sustained soldier body weight during extended periods of field ration use. During

the first 5 days of field operations, only the first module would be issued. If the operation was more prolonged, hot group feeding would be instituted at day 5 or the first and second modules would be issued together.

CONCLUSIONS

1. In the short term, the fat content of rations has little positive or negative effect on either physiologic responses or physical performance of moderately active soldiers.

2. Short term energy deficits can be readily met by using body fat stores.

3. A dietary carbohydrate intake of 300 g/day is insufficient to prevent a transition from a carbohydrate- to a fat-predominant metabolism during four days of moderate exercise.

RECOMMENDATIONS

1. Set as a development goal for packaged rations the consumption of 400 to 440 g of carbohydrate per day.

2. Deliver this amount of carbohydrate in conjunction with 70 g protein and as much fat as practical within weight and volume constraints.

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APPENDIX A

NUTRITIONAL SUSTAINMENT MODULE FACT SHEET, MENUS AND NUTRIENT COMPOSITION TABLE

Technology Acquisition
and Development Branch
Food Technology Division
Food Engineering Directorate

FACT SHEET

SUBJECT: Nutritional Sustainment Module (NSMs), a New Ration for Military Use

FACTS:

1. The NSMs represent a new concept in military feeding initiated by TRADAC to meet the combat needs of ARMY 21. Emerging combat doctrines and scenarios require military units to be highly mobile and dispersed in depth behind enemy lines without the possibility of resupply for long periods of time. As projected by TRADOC, the ration's distinctive characteristics are:

a. *It must* be of the highest possible caloric density, approaching 7.1 kcal/cc; this compactness would allow a dismounted soldier to carry easily a 3-5 day supply of food meeting the 3600-kcal daily requirement.

b. It must be nutritionally adequate to maintain physical and mental performance under highly stressful operational conditions; it should be supplemented with a variety of natural and/or synthetic food components to ensure maximum caloric utilization and maximum nutritive value.

c. It must be acceptable to the consumer, despite its unfamiliar nature, throughout the period of use, which might even exceed 5 days.

d. It must be of optimum utility, consumable on the move without any preparation, and modular in design; for example, a 900-kcal module should be combinable to form rations of 1800, 2700, 3600, and 4500 kcal/day to meet specific combat scenario requirements.

2. The Natick RD&E Center is coordinating with OTSG, TRADOC, and the other Services in refining the NSM concept with respect to the projected length of use, limits on fat content, caloric density, nutrient composition, and supplementation.

3. Accomplishments to date using ICE Technologies (Infusion, Compression, Extrusion) are:

a. Demonstrated feasibility of infusing high melting fats into the open matrices of dry foods and extruded foods. Several infusion liquids (cheese, yogurt, chocolate, peanut butter, etc.) with about 55% fat, 25% carbohydrate, 15% protein, and 5% flavorings have been developed and infused into freeze-dried meats, vegetables, and extruded products.

b. Developed meat analogues which serve as matrices for the infusion of flavored oils. Meat entree products reaching 7 kcal/cc were developed using this technology.

c. Families of compressed dairy bars, chowder entree bars, and cocoa and mocha beverage bars have been developed demonstrating a wide variety of products that achieve high caloric densities with high acceptance ratings.

d. A high temperature short time (HTST) cooking extruder has been used to demonstrate the feasibility of extruding highly nutritional, carbohydrate-based matrices for infusion. Extrusion of cheese-based and chocolate-based products has been demonstrated. Extrusion allows the fabricating and controlling of texture, cell structure, volume, and nutrition in food materials.

e. High caloric dairy and cocoa bars received scores of 7-8 (9=excellent Hedonic scale) when field-tested as components in the RLW30 ration.

f. Conducted a field test on a NSM 900 kcal module. The rations strong points were that it is compact, light, easy to carry, easy to open and consume. The test indicated a need to reduce powdery texture, to provide more texture, to improve the entree-type products and make provisions for rehydrating.

g. A module demonstrator has been developed with the following characteristics: 900 kcal, 160 cc, 150 g, 5.6 kcal/cc, and 6.0 kcal/g. This demonstrator module exceeds the design criteria for caloric density and represents an 86% reduction in the cube and 70% reduction in weight over the current MRE.

h. Scanning Electron Microscopy (SEM), Mercury Intrusion Porosimety, Enzymatic Gelatinization Tests, Mechanical Property Analysis, and Light Scattering Particle Size Analysis have been employed to determine product characteristics such as extrudate pore size, volume,

degree of cook, particle size, and textural properties. These analyses have helped to establish process controls.

i. Correlations are being developed between sensory subjective analysis and chemical/physical analysis. Also marketing techniques are being explored to optimize the presentation and the use of unfamiliar foods.

Approved Irwin A. Taub
Branch Chief

DIET: 2300 KCAL, DAY 1					
ITEM	**WT (g)**	**KCAL**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Total Cereal Bar	60	280.4	4.51	12.72	36.97
Strawberry Dairy Bar	20	119.9	1.65	8.57	9.05
Western Omelet Carbo Cr	60	255.0	7.99	11.15	30.65
Peach Punch Crunch, LO	15	49.6	.52	.12	11.62
SUBTOTALS	**155**	**704.9**	**14.67**	**32.56**	**88.29**
SNACK					
Fruit Chew, Grape	40	152.0	.49	2.79	31.24
Cocoa Bar	30	247.8	3.68	19.54	14.30
SUBTOTALS	**70**	**399.8**	**4.17**	**22.33**	**45.54**
LUNCH					
Beef Stick	20	87.04	8.74	5.32	1.05
Roast Pork Carbo Crisp	40	168.6	7.15	7.23	18.72
Fruit Chew	30	114.0	.37	2.09	23.43
Crm Tomato Chowder	30	177.8	3.20	13.98	9.80
Coconut Punch Cr, LO	30	99.2	1.03	.24	23.23
SUBTOTALS	**150**	**646.6**	**20.49**	**29.02**	**76.23**
Dinner					
Pork & Rice	100	464.3	29.33	19.36	43.21
Raspberry Punch Cr, LO	15	99.20	1.03	.24	23.23
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**145**	**677.5**	**30.73**	**21.69**	**89.87**
TOTALS	**520**	**2428.8**	**70.06**	**105.6**	**299.93**

DIET: 3300 KCAL, DAY 1					
ITEM	**WT (g)**	**KCAL**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Total Cereal Bar	90	420.6	6.77	19.08	55.46
Strawberry Dairy Bar	35	229.5	2.83	18.88	12.07
Western Omelet CC	25	89.9	2.54	11.16	8.77
Banana Punch Crunch, low	20	66.2	.69	.16	15.49
SUBTOTALS	**170**	**806.2**	**12.83**	**49.28**	**91.79**
SNACK					
Fruit Chew	30	114.0	.37	2.09	23.43
Cocoa Bar	40	247.8	3.68	19.54	14.30
Raspberry Punch Cr, HI	35	218.2	.63	17.77	13.93
SUBTOTALS	**105**	**591.4**	**4.71**	**39.40**	**54.01**
LUNCH					
Beef Stick	30	130.5	13.12	7.97	1.57
Roast Pork, CC	25	154.8	3.80	12.86	5.97
Fruit Chew, Cherry	30	114.0	.37	2.09	23.43
Crm Tomato Chowder	80	510.0	7.74	43.84	21.12
Orange Punch Crunch, LO	20	66.2	.69	.16	15.49
SUBTOTALS	**165**	**986.9**	**25.75**	**67.13**	**69.93**
DINNER					
Pork and Rice	70	325.1	20.53	13.55	30.25
Mixed Nut Dairy Bar	35	242.8	3.63	21.52	8.65
Coconut Punch Crunch, HI	35	218.2	.63	17.77	13.93
Fruit Chew, Strawberry	30	114.0	.37	2.09	23.43
SUBTOTALS	**170**	**911.5**	**25.19**	**55.14**	**78.61**
TOTALS	**610**	**3296.0**	**68.48**	**210.95**	**294.34**

DIET: 2300 KCAL, DAY 2					
ITEM	WT (g)	KCAL	PRO (g)	FAT (g)	CHO (g)
BREAKFAST					
Bran Cereal Bar	80	374.5	7.43	17.82	46.10
French Toast CC	50	209.5	5.40	8.59	27.65
Orange Punch Crunch, LO	25	82.7	.86	.20	19.36
SUBTOTALS	**155**	**666.7**	**13.69**	**26.61**	**93.11**
SNACK					
Fruit Chew, Strawberry	30	114.0	.37	2.09	23.43
Cocoa Bar	25	154.8	2.30	12.21	8.94
SUBTOTALS	**55**	**268.8**	**2.67**	**14.3**	**32.37**
LUNCH					
Pork Stick	30	140.1	12.08	9.34	1.94
Pizza, CC	50	202.8	5.36	7.92	27.51
Fruit Chew, Cherry	30	114.0	.37	2.09	23.43
Banana Punch Crunch, LO	25	82.7	.86	.20	19.36
SUBTOTALS	**135**	**539.6**	**18.67**	**19.55**	**72.24**
DINNER					
Chicken and Rice	90	399.6	29.66	13.39	40.10
Bacon and Corn Chowder	30	175.3	2.55	13.19	11.60
Banana Walnut Dairy	20	127.8	1.92	10.09	7.32
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**170**	**816.7**	**34.50**	**38.76**	**82.45**
TOTALS	**515**	**2291.8**	**69.53**	**99.22**	**280.17**

DIET: 3300 KCAL, DAY 2					
ITEM	**WT (g)**	**KCAL**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Bran Cereal Bar	90	421.2	8.36	20.04	51.86
Banana Walnut Dairy Bar	35	237.9	3.22	20.53	10.05
French Toast Carbo Cr	25	143.7	1.73	10.74	10.02
Strawberry Punch Cr, LO	20	66.16	.69	.16	15.49
SUBTOTALS	**170**	**869.0**	**14.0**	**51.47**	**87.42**
SNACK					
Fruit Chew	30	114.0	.37	2.09	23.43
Cocoa Bar	45	278.7	4.14	21.98	16.08
Peach Punch Cr, HI	30	187.0	.54	15.23	11.94
SUBTOTALS	**105**	**591.1**	**5.08**	**39.51**	**53.8**
LUNCH					
Pork Stick	30	140.1	12.07	9.34	1.94
Pizza Carbo Crisp	25	150.7	2.01	12.34	7.91
Fruit Chew, Grape	30	114.0	.37	2.09	23.43
Bacon & Corn Chowder	80	501.14	6.53	41.70	24.93
Strawb.-Banana Punch, LO	20	66.16	.69	.16	15.49
SUBTOTALS	**185**	**983.5**	**21.70**	**65.84**	**76.05**
DINNER					
Chicken & Rice	70	310.7	23.06	10.42	31.18
Vanilla Dairy Bar	35	242.1	3.35	21.68	8.40
Cherry Punch Cr, HI	30	187.0	.54	15.23	11.94
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**165**	**865.2**	**27.35**	**49.63**	**77.30**
TOTALS	**625**	**3308.8**	**68.13**	**206.45**	**294.57**

DIET: 2300 KCAL, DAY 3					
ITEM	**WT (g)**	**KCAL**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Life Cereal Bar	100	487.27	11.34	23.19	58.30
Ham & Egg Carbo Crisp	50	313.41	6.25	26.65	12.14
Punch Crunch, LO	30	99.20	1.03	.24	23.23
SUBTOTALS	**180**	**899.9**	**18.62**	**50.08**	**93.67**
SNACK					
Fruit Chew	40	152.03	.49	2.79	31.24
Cocoa Bar	20	123.89	1.84	9.77	7.15
SUBTOTALS	**60**	**275.9**	**2.33**	**12.56**	**38.39**
LUNCH					
Beef Stick	20	87.04	8.74	5.32	1.05
Beef Taco Carbo Crisp	50	160.98	7.14	3.82	24.51
Fruit Chew	30	114.0	.37	2.09	23.43
Seafood Chowder	30	184.38	2.87	13.90	10.95
Punch Crunch, LO	30	99.20	1.03	.24	23.23
SUBTOTALS	**160**	**657.0**	**20.18**	**25.58**	**85.52**
DINNER					
Spaghetti	70	244.83	22.00	14.55	26.27
Almond Dairy Bar	20	123.72	2.70	9.44	6.99
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**120**	**493.9**	**25.10**	**26.29**	**59.04**
TOTALS	**520**	**2326.7**	**66.23**	**114.51**	**276.62**

DIET: 3300 KCAL, DAY 3					
ITEM	**WT (g)**	**KCAL**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Life Cereal Bar	90	438.5	10.21	20.87	52.47
Almond Dairy Bar	35	230.18	4.84	19.18	9.55
Ham & Egg CC	25	156.77	3.13	13.33	6.07
Strawberry Punch Cr, LO	20	66.16	.69	.16	15.49
SUBTOTALS	**170**	**891.6**	**18.87**	**53.54**	**83.58**
SNACK					
Fruit Chew	30	114.0	.37	2.09	23.43
Cocoa Bar	40	247.78	3.68	19.54	14.30
Orange Punch Cr, HI	30	187.0	.54	15.23	11.94
SUBTOTALS	**100**	**560.2**	**4.62**	**37.07**	**52.02**
LUNCH					
Beef Stick	25	108.07	10.61	6.71	1.31
Beef Taco, Carbo Cr	40	238.57	4.59	19.73	10.66
Fruit Chew, Cherry	40	152.03	.49	2.79	31.24
Seafood Chowder	75	490.41	7.16	42.01	20.92
Banana Punch Cr, LO	20	66.16	.69	.16	15.49
SUBTOTALS	**200**	**1055.2**	**23.54**	**71.40**	**79.62**
DINNER					
Spaghetti	60	277.75	18.86	12.47	22.52
Orange/Pineapple Dairy	35	235.27	2.83	19.95	11.10
Peach Punch Cr, HI	30	187.0	.54	15.23	11.94
Fruit Chew, Strawberry	30	114.0	.37	2.09	23.43
SUBTOTALS	**155**	**825.4**	**22.63**	**49.95**	**71.34**
TOTALS	**625**	**3332.4**	**69.66**	**211.96**	**286.56**

DIET: 2300 KCAL, DAY 4					
ITEM	**WT (g)**	**KCAL (g)**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Wheaties Cereal Bar	80	380.5	7.15	17.82	47.89
Cheese Carbo Cr	50	219.80	7.28	11.17	22.54
Punch Crunch, LO	30	99.20	1.03	.24	23.23
SUBTOTALS	**160**	**699.5**	**15.46**	**29.23**	**93.66**
SNACK					
Fruit Chew	40	152.0	.49	2.79	31.24
Cocoa Bar	20	123.9	1.84	9.77	7.15
SUBTOTALS	**60**	**275.9**	**2.33**	**12.56**	**38.39**
LUNCH					
Pork Stick	25	116.7	10.06	7.78	1.61
Oriental Chicken CC	50	221.9	16.47	7.44	22.27
Fruit Chew	30	114.0	.37	2.09	23.43
Punch Crunch, LO	30	99.20	1.03	.24	23.23
SUBTOTALS	**135**	**563.2**	**27.96**	**17.76**	**72.89**
DINNER					
Pork and Rice	80	371.5	23.46	15.49	34.57
Bacon & Corn Chowder	20	116.8	1.70	8.79	7.73
Almond Dairy Bar	30	185.6	4.05	14.17	10.48
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**160**	**799.3**	**29.61**	**40.75**	**78.56**
TOTALS	**515**	**2337.9**	**75.36**	**100.3**	**283.5**

DIET: 3300 KCAL, DAY 4					
ITEM	**WT (g)**	**KCAL (g)**	**PRO (g)**	**FAT (g)**	**CHO (g)**
BREAKFAST					
Wheaties Cereal Bar	90	428.0	8.05	20.04	53.87
Strawberry Dairy Bar	35	229.5	2.83	18.88	12.07
Cheese Carbo Crisp	25	131.6	2.27	12.82	7.19
Coconut Punch Cr, LO	20	66.2	.69	.16	15.49
SUBTOTALS	**170**	**855.3**	**13.84**	**51.9**	**88.62**
SNACK					
Fruit Chew	30	114.0	.37	2.09	23.43
Cocoa Bar	40	247.8	3.68	19.54	14.30
Cherry Punch Cr, HI	35	218.2	.63	17.77	13.93
SUBTOTALS	**105**	**591.4**	**4.71**	**39.61**	**54.01**
LUNCH					
Pork Stick	30	140.1	12.07	9.34	1.93
Oriental Chicken Stick	25	111.0	8.24	3.72	11.14
Fruit Chew, Grape	30	114.0	.37	2.09	23.43
Bacon & Corn Chowder	80	501.2	6.53	41.70	24.95
Strawb/Banana Punch Cr	20	66.2	.69	.16	15.49
SUBTOTALS	**185**	**943.9**	**27.93**	**57.22**	**79.29**
DINNER					
Pork & Rice	70	325.1	20.53	13.55	30.25
Almond Dairy Bar	35	230.2	4.84	19.18	9.55
Raspberry Punch Cr, HI	30	187.0	.54	15.23	11.94
Fruit Chew	30	114.0	.37	2.09	23.43
SUBTOTALS	**165**	**867.7**	**26.31**	**50.26**	**77.52**
TOTALS	**625**	**3258.3**	**72.79**	**198.99**	**299.44**

APPENDIX B

ACCEPTABILITY OF NUTRITIONAL SUSTAINMENT MODULE DEMONSTRATION RATION

ACCEPTABILITY OF NUTRITIONAL SUSTAINMENT MODULE STUDY RATIONS

ACCEPTANCE MEASUREMENT

Ration Acceptance Questionnaires

Each subject recorded daily what he liked and disliked about the categories of ration components using a nine-point hedonic scale (1= "dislike extremely," to 9 = like extremely") for each ration component. The subject's subjective opinions of the rations were addressed through questions such as "How do you feel that this ration affected your overall performance?", and "If you had only this ration to eat in combat, how many days would it have sustained you?" with responses on a 7-point scale (1 = extremely positive effect," to 7 = "extremely negative effect"). The subjects were also asked how the ration might be improved through questions such as: "If changes were to be made to the rations that you ate during this test, what characteristics of the ration would you most want to see changed?" (easier to open, less thirst, taste better, more variety, more filling, not crumble, rehydrate faster, etc.) with the responses ranked from "1" as the most important, "2" as the next important, etc., and "If you could design your own daily ration using the same packages of bars as you had, and you had 10 bars available, how many of each type of package would you want per day?." Mean satiety ratings were calculated for each ration. In addition, there were questionnaires at the completion of each exercise/diet week in which the subjects were asked to assess ration acceptability and suggest ways to improve the rations.

Questionnaire Design

Separate questionnaires were designed for each of the three mealtimes and the morning snack period for both the 2300 and 3300 kcal diets. In the sample questionnaires, the test day, meal, and diet were encoded for reference of data collectors. Food names were preprinted for all except the punch crunch items for which the flavor types were unknown until an individual menu was assembled. Subjects rated acceptability using a modified version of the 9-category hedonic scale; due to space limitations with a linear layout, only the scale end and middle word descriptions were displayed. Subjects were also asked to indicate whether or not they rehydrated those foods that were rehydratable. At the end of the questionnaire, allowance was made for additional comments. Questionnaires were designed to be self-administered; however, data collectors were on hand to assist, if clarification was necessary.

Meals

During the Monday through Thursday test periods, subjects were not allowed to consume any foods other than the NSM components. The diets the subjects consumed were purposely formulated using developmental components from both the NSM and the Ration Lightweight to acheive a difference in fat content that could not be readily detected by subjects eating side by side. Bringing snacks, etc., was strictly forbidden. However, unlimited liquids, such as water, and decaffeinated coffee, tea, and carbonated diet beverages, were allowed. Unless medical or physiological reasons arose, subjects were strongly encouraged by data collectors to eat everything provided. To monitor caloric intake, leftover portions were weighed and entered on a Diet Record unless a subject would eat them later.

Administration of questionnaires

Food items for each mealtime were assembled and packaged in polymeric olive drab color mealbags. Prior to the mealtime, the appropriate questionnaires for that meal and diet were stapled to each subject's mealbag. Before eating or rating any food, subjects were asked to indicate, on the first rating scale, how hungry they were. They then prepared (rehydrated) any items they wished to rehydrate with the hot water provided and proceeded to eat.

Following the dinner mealtime on Thursday of each of the four test weeks, subjects were administered a posttest questionnaire. In it, they were asked to rate, from memory, the food items they had eaten during the test period. In addition, they were asked: to react to salient characteristics of the ration, to select a preferred daily "menu" from the product classes they had eaten, to react to portion sizes/quantity provided at each mealtime, and to comment on what foods should be added to or eliminated from the menu.

Focus Group Discussion

Two weeks after the conclusion of the study, four of the eight subjects participated in a focus group discussion of their experiences with the NSM components. Two subjects were military laboratory technicians and two were test subjects recruited specifically for the present and other tests. The discussion was represented as an informal debriefing in which subjects could share their comments, ideas, questions, and perceptions about the products on which they had subsisted during the tests. Five objectives were set for the discussion: (1) to elicit subjects' general impressions/opinions of each of its main components (they had subsisted on the ration

for two four-day periods, separated by a week of garrison A rations), (2) to discover participants' attitudes about nutrition and product labeling when projected to a field scenario, (3) to determine the importance of nutrition to participants versus eating their favorite foods, (4) to learn what food cravings participants had during the test periods, and (5) to receive suggestions on appropriate names for the main ration components.

RESULTS

Based on fat level of NSM components, there were two types of products: (1) those presented to subjects at a "low" fat level in the 2300 kcal diet and a "high" fat level in the 3300 kcal diet (punch crunch, chowders, dairy bars, and carbo crisps), and (2) those that were of identical composition in both diets (meat sticks, cocoa bars, cereal bars, entree bars, and fruit chews).

Because of the small number (4) of subjects participating in the study each of the four weeks, from eight to 16 ratings were normally obtained per product per diet, depending on the frequency served (one to four times during the week). Since this yielded an insufficient number of ratings per items to be statistically definitive, the data were combined across all varieties of nine product groups according to the Calorie level of the diet.

The bar graphs in Figure 1 indicate acceptability, as measured with the 9-category hedonic scale, for the nine product groupings by normal (2300 kcal) and added fat (3300 kcal) diets. Except for dairy bars, as indicated below, differences were inconsequential, both for those products that differed in fat level and those that were identical in composition.

PRODUCT GROUPS VARYING IN FAT LEVEL

Of the four product groups having low and high fat levels, the first were punch crunch bars (7 varieties). Mean ratings were near 3.0 (dislike moderately) at both fat levels, and standard deviations approached the mean (2.6 and 2.7, respectively). This wide deviation occurred because two or three subjects in each group of eight ratings indicated a high degree of liking for the items while the others expressed extreme dislike. Ratings by flavor ranged from 2.2 to 4.1 and varied randomly between low and high fat versions of the same flavor variety. These were the lowest rated of the NSM components. Written comments from rating forms and the follow-up focus group session indicated that the products were too moist, too oily, mushy (this is probably due to attempts to rehydrate items not intended for rehydration), or scratchy and

gritty. Flavors were considered much too artificial and strong. From the product name, subjects were possibly expecting that they could prepare a beverage from the bars.

The three varieties of chowder bars were the most acceptable of the low and high fat product families, rating 7.0 (like moderately) or above. There was a small difference between low and high fat versions. Seafood chowder was more highly rated than the bacon-corn or spicy garden vegetable versions. These products appeared to be an ideal medium for carrying fat calories into the diet since fat is inherent to the items.

Lower fat versions of the dairy bars were more highly rated (6.3 - like slightly) than the higher fat versions (5.2 - neither like nor dislike). This was the greatest difference found between product groups with different fat levels. It was not explainable by comments on ratings forms or from the focus group discussions. Ratings for three varieties of the low fat products served with the 2300 kcal diet ranged from 5.5 to 6.6, while for the six higher fat products served with the 3300 kcal diet the range was 4.4 to 5.9.

The lower fat varieties of the carbo crisp group were rated the same as the high fat varieties (overall, 5.3 - neither like nor dislike), and there was considerable scatter in the ratings, as was noted from the standard deviations (2.6 and 2.1, respectively). Although the "high fat" type was considered more palatable than the "dry type," it was evident that neither were well received. Ratings for individual varieties/flavors ranged from 2.4 for the high fat "western omelet" to 6.8 for the high fat "beef taco." Both questionnaire and focus group comments indicated that all varieties had strong odors but inadequate flavoring, the high fat versions "repeated" on subjects during exercise, and the dry types were like cardboard. Some found the items to be thirst producing.

FOODS IDENTICAL IN COMPOSITION

Five product types were included in this group: meat sticks, cocoa bar, cereal bars, entree bars, and fruit chews. Three product groups each had three varieties/flavors, the cereal bars which had four varieties, and the cocoa bar was a single item. All product groups and individual varieties rated 7.0 (like moderately) or higher. From focus group comments, it was evident that, common to each of these product groups, was the fact that they were familiar foods, either when eaten "as is" or rehydrated for eating. Highest rated of the product groups were all three entree bar varieties (8.0 - like very much). Almost as highly rated were the three varieties of meat sticks which, like the chowder bars, appeared to be an appropriate medium for conveying fat

calories to NSM diets. Cereal bars, although highly rated, received comments that colors and related fruit flavors would give the products more interest and improve the mood of troops eating them. Finally, the three fruit chews flavors and the cocoa bar received equivalent ratings in both diets.

ADDITIONAL COMMENTS FROM FOCUS GROUP DISCUSSION

Carbo Crisps

The consensus was that the 'greasy crisps' were more palatable than the dry type, but neither type was well received. The most disliked flavor was the WESTERN OMELET. Greasy types repeated on respondents during exercise, and the dry types were referred to as 'cardboard'. Odors were strong (FRENCH TOAST, PIZZA, WESTERN OMELET) and appealing but with inadequate flavoring. The name 'carbo crisp' was found to be fitting but with a negative connotation, i.e., carbon describes coal. Subsistence on carbo crisps would be strictly for survival and would cause respondents to seek other alternatives for food.

Chowder Bars

The consensus was favorable on the concept of chowder bars, and the preferred flavors were SEAFOOD and BACON-CORN. The SPICY VEGETABLE was least liked. One favorable aspect was the importance of having a hot, hearty (textured) soup in the field in cold weather. Two out of four subjects rehydrated the bars. The impracticality of having to rehydrate a food in hot water during combat was discussed. Additional flavors such as bean and barley or others with meat bases were recommended. The name CHOWDER BAR is suitable unless other flavors are added, in which case the name SOUP BARS was recommended.

Punch Crunch

The overall consensus was very unfavorable (see previous discussion).

Cocoa Beverage Bars

All four subjects would not change this product. One suggestion was to add caffeine to give a boost during extended duty. Additional flavors were also suggested: eggnog, tea, mocha.

This product was well liked and referred to as a 'treat'. Although this bar is high in fat, the fat level was not objectionable.

Meat Sticks

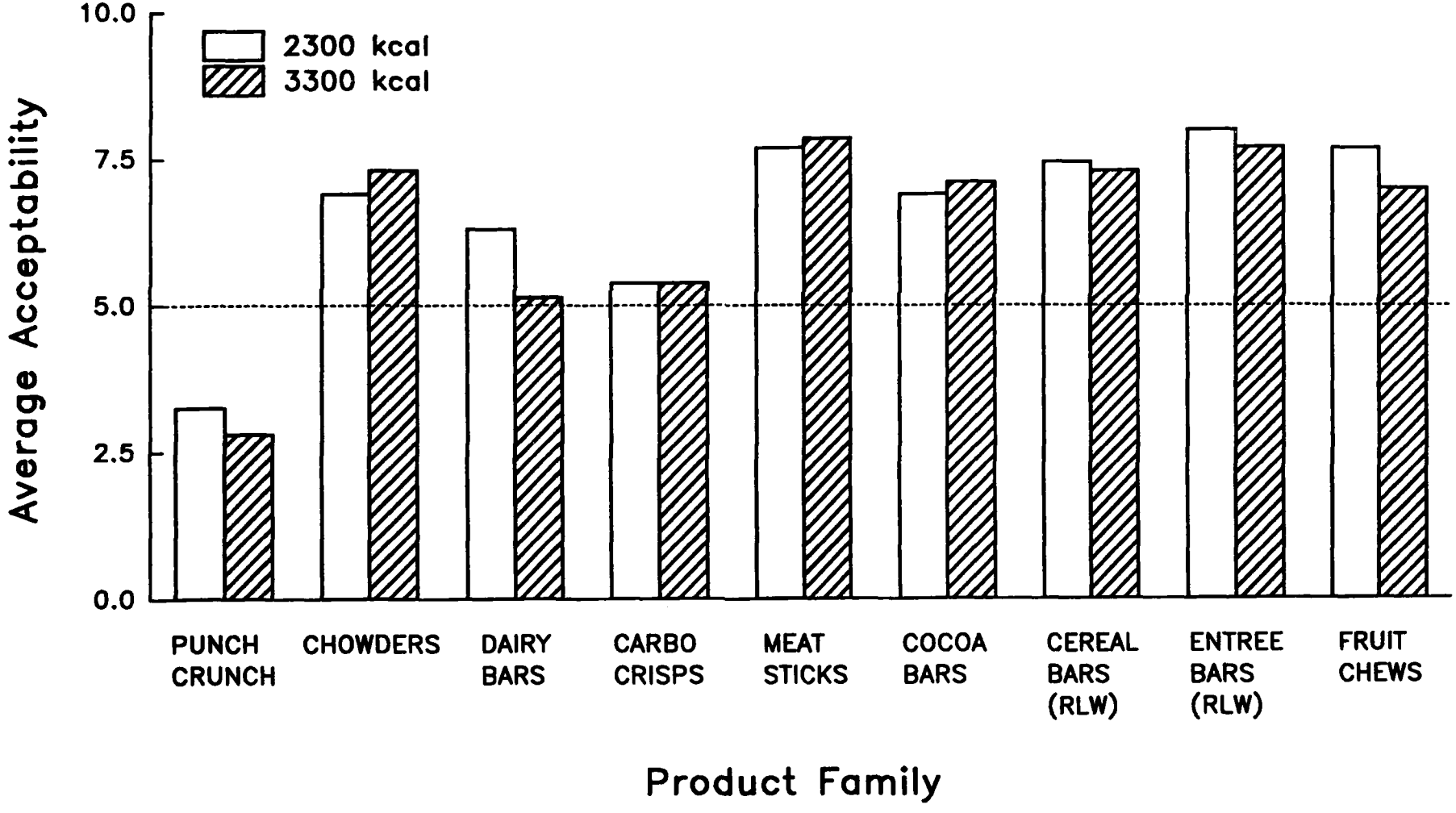
All four panelists rated this product as excellent. One described it as 'a piece of home'. The consensus was that it is important to have familiar foods in a field scenario. Recommendations included: combining potato sticks with the meat sticks, develop turkey, sausage, and chicken varieties. All commented that they expected fat in these products and therefore did not find the fat present objectionable. In general there was no objection to foods infused with fat when they normally contain fat. Objections arose when an unfamiliar food is infused with fat.

Cereal Bars

The panelists felt that this product was acceptable and made the following observations: more flavors, particularly sweeter flavors, should be added (eg. FRUIT LOOPS and CAPTAIN CRUNCH); the bars lacked color and resembled brown bags or cardboard; hot cereals such as oatmeal should be made into bars.

Due to time constraints the FRUIT CHEWS or the ENTREE BARS were not discussed.

Figure 1


10.0
7.5
5.0
2.5
0.0
2300 kcal
3300 kcal
Average Acceptability
PUNCH CRUNCH
CHOWDERS
DAIRY BARS
CARBO CRISPS
MEAT STICKS
COCOA BARS
CEREAL BARS (RLW)
ENTREE BARS (RLW)
FRUIT CHEWS
Product Family

APPENDIX C

IMPACT OF A HIGH-FAT DIET ON THE FECAL MICROFLORA OF MALE ADULT SUBJECTS

IMPACT OF A HIGH-FAT DIET ON THE FECAL MICROFLORA OF MALE ADULT SUBJECTS

ABSTRACT

The effect of a high-fat, calorically dense military ration (Demonstration Nutritional Sustainment Module) on the intestinal microbiota and the bacterial β-glucuronidase activity was studied in young healthy male military subjects. The results indicated that neither the high-fat (57% of Calories) nor the lower-fat (40% of Calories) NSM ration altered the intestinal microflora over the four day experimental period. However, the bacterial β-glucuronidase activity did increase when the test subjects were placed on the high-fat diet. Thus the fecal microflora of subjects eating high-fat NSM rations were more able to hydrolyze glucuronide conjugates.

INTRODUCTION

The Nutritional Sustainment Module (NSM), a new combat ration concept, is a calorically dense (high fat) operational ration. Since a significant proportion of its caloric content is contributed by fat (39 - 57%), there is concern about possible short-term adverse health effects (e.g., gastrointestinal disturbances) that might result from its consumption.

The specific microbiological interest in the NSM ration is the potential of its high-fat content to alter the intestinal microflora. Shifts in the intestinal microflora, were they to occur, could cause major physiologic disturbances, e.g., acute diarrhea, or, if prolonged, cirrhosis (Simon and Gorbach, 1984). However, there is conflicting data in the literature regarding the ability of the diet to alter specific microbial components of the adult human fecal microflora (Gorbach, 1986; Goldin, 1986). Moore and Holdeman (1975) found no changes in the fecal microflora of individuals shifted from an omnivorous to a vegetarian diet. On the other hand, studies by Maier et al. (1974) and Reddy et al. (1974) suggest that high-meat diets (i.e., with higher fat contents) do cause shifts in components of the fecal microflora. Drasar et al. (1976) conducted studies on the effect of high fiber diets on the fecal microflora. They reported no change in the composition of fecal microflora as a result of diet modification. The most popular held belief today is that diets can cause overall shifts in the fecal microflora, but the alterations are not significant, e.g., gross changes in the composition of intestinal flora do not occur (Goldin, 1986).

The complexity of the fecal microflora that can be present (> 400 species) presents quantitation and taxonomic difficulties for showing changes in population in response to varying diets. Another approach is to measure the metabolic activity of the microflora in relation to specific substrates. Gorbach (1986) and Goldin (1986) have reported metabolic activity to be a sensitive measure of microbial activity and more responsive to changes in diet. It has, in fact, been reported that diet can, indeed, alter the metabolic activity of the intestinal flora (Goldin, 1986).

The present study was designed to ascertain relevant information about possible changes in the microbiota of the feces of young active military subjects maintained on model NSM rations that were either high fat/3300 kcal or lower fat/2300 kcal (protein and carbohydrate were kept constant). The specific objectives of the microbiological study were:

1. To profile the normal intestinal microflora of each test subject.
2. To characterize any changes in the fecal microflora of the test subjects during the experimental period of five days.
3. To determine the metabolic activity (β-glucuronidase) of the fecal microflora.

MATERIALS AND METHODS

STUDY POPULATION

The following criteria were used in selecting test subjects: age, <35 years; sex male; no antibiotic use within 2 to 3 weeks of the study; free of any gastrointestinal diseases (diarrhea, constipation or bowel disease); and on no special diet prior to the study. The compositions of experimental diets are shown in Table 2 on page 5 of the main report. Each diet was evaluated over a one week period. The diet schedule was given as described in Table I.

Table I
Test Diet Schedule

A = Basal Diet Plus Added Fat B = Basal Diet		GROUP							
		I				II			
		Subject				Subject			
Week	Diet	1	2	3	4	5	6	7	8
1	A	X	X						
	B			X	X				
2	A					X	X		
	B							X	X
3	A			X	X				
	B	X	X						
4	A							X	X
	B					X	X		

Table II
Sampling Schedule

week	Su	Mo	Tu	We	Th	Fr	Sa
1					S[a] G-I[b]	S[a] G-I[b]	
2				S G-II	S G-II		
3				S G-I	S G-I		
4				S G-II	S G-II		

[a]Sample collected. Sampling period began at 6 a.m. on indicated day and ended the following day at 6 a.m.
[b]G-I and G-II. The eight test subjects were divided into two groups, i.e. four individuals per group.

COLLECTION AND PREPARATION OF FECAL SPECIMEN

Fecal samples were collected in clean plastic bags and stored at 1-5°C for no longer than 24 hours (Gorbach et al., 1967). The sampling schedule was as outlined in Table II.

QUANTITATION OF FECAL MICROFLORA

The methods used to quantitate the microflora were similar to the methods used by Gerhardt and Iglewski (1976), with the following modifications: (1) each 24 hours composite stool was blended by placing the entire fecal sample in a sterile Stomacher bag (Tekmar, Cincinnati, OH; 400 ml capacity) and blending thoroughly for 1-2 minutes, (2) a 1 g aliquot of the blended feces was added to 9 ml of sterile diluent (KH_2PO_4. 4.5 g; Na2PO4, 6 g; 1 Cysteine-HCL, 0.3 g; Tween 80, 1 g; Agar, 1 g; distilled water, 1000 ml; pH 7.3)(Ueno et al., 1974) and dispersed by vortexing for 30 seconds, and (3) serial 10-fold dilution were made, and duplicate 0.1 ml of each dilution was spread onto prepoured plates of specific selective media.

The procedures for enumerating and isolating fecal microflora proceeded as described in Table III.

Table III Bacterial Isolation Procedures			
BACTERIA		INCUBATION CONDITIONS	MEDIA
Total aerobic		37°C, aerobic, 2 days	Blood agar
	Lactobacilli	" " "	LBS agar
	Streptococci	" " "	Bile Esculin Azide agar
Total anaerobic		37°C, anaerobic, 2 days	CDC Anaerobic Blood agar
	Bacteroides	" " "	Bacteroides Bile Esculin agar
	Fusobacterium & Eubacterium	" " "	Rifampin Blood agar
	Clostridium	" " "	McClung & Toabe agar, modified

BETA-GLUCURONIDASE ASSAY PROCEDURES

After thoroughly mixing the entire 24 hour fecal sample, 1 g quantities were removed and placed in sterile Stomacher bags (Tekmar Co. Inc., Cincinnati, OH). Samples were then placed on ice, where they remained until fecal extracts were prepared. The procedure described by Goldin et al. (1980) was used for the preparation of extracts and enzyme assays with the following modifications: Ten ml of 0.1 M potassium phosphate (pH 7.0) was added to 1 g aliquots of the feces and blended (Stomacher; Tekmar Co., Inc. Cincinnati, OH) for 30 seconds. After blending, fecal samples were subjected to sonic disruptions (Bronson Sonifier 350, Bronson Sonic Power Co., Danbury, CT), with eleven 30 second pulses with 1 to 1.5 minute interpulse periods for cooling (<4°C). Samples were then centrifuged in a RC-5 Superspeed refrigerated centrifuge (Du Pont Co., Bear, DE) at 500 x g for 15 minutes, and the supernatants were decanted and filtered through cellulose membrane filters (5 micron; Microfiltration Systems, Dublin, CA). The final filtrate was used for the enzyme assay. All enzyme assays were run by Dr. Barry Goldin, Tufts-New England Medical Center Hospital, Boston, MA.

Table IV

The Fecal Flora of Subjects Consuming Model Nutritional Sustainment Modules

Diet A: Basal diet plus added fat Diet B: Basal diet

Organism	Test Subject Log[10] Numbers of Viable Cells/g Feces							
	1	2	3	4	5	6	7	8
Total Aerobic								
Control	9.1	9.3	<7.0	<7.0	<5.0	<6.0	nd[a]	<6.0
Diet A	6.1	7.3	<6.0	<5.0	<6.0	<6.0	7.5	<6.0
Diet B	<6.0	<6.0	6.2	<6.0	0.7	7.1	<6.0	<7.0
Lactobacilli								
Control	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	nd	<5.0
Diet A	<5.0	<5.0	5.6	<5.0	5.7	<4.0	<5.0	5.3
Diet B	5.3	4.5	4.5	5.5	5.7	<4.0	5.6	4.5
Streptococci								
Control	<5.0	<5.0	<5.0	<5.0	6.8	<5.0	nd	<5.0
Diet A	6.4	6.1	<5.0	6.5	6.7	<5.0	6.0	<5.0
Diet B	<5.0	6.7	6.2	6.3	7.5	<4.0	6.6	5.2
Total Anaerobic								
Control	<7.0	8.3	10.4	10.8	9.7	10.0	nd	9.8
Diet A	10.0	7.6	10.0	10.2	10.0	10.2	9.7	9.9
Diet B	<8.0	10.0	10.0	9.4	9.5	9.7	9.0	9.6
Bacteroides								
Control	<7.0	<7.0	10.5	9.7	9.6	10.2	nd	9.7
Diet A	9.7	9.6	10.2	10.0	10.0	10.0	9.6	9.7
Diet B	10.0	9.5	10.0	9.7	9.4	9.8	9.8	9.6
Clostridia								
Control	8.8	9.1	10.6	9.3	9.5	10.0	nd	9.7
Diet A	9.9	8.8	10.0	9.2	9.8	9.7	9.3	9.5
Diet B	9.8	9.5	6.5	9.5	8.5	7.0	9.0	<6.0
Fusobacteria & Eubacteria								
Control	<7.0	<7.0	9.0	9.0	9.7	9.7	nd	8.4
Diet A	8.5	9.6	<6.0	<7.0	6.6	7.7	7.3	<6.0
Diet B	7.5	6.7	5.8	<6.0	<5.0	<5.0	<6.0	<5.0

[a]No microbiological analysis was done.

RESULTS AND DISCUSSION

The results appear to be consistent with observations made by Goldin (1986) and Simon and Gorbach (1984) that anaerobic bacteria (e.g., Bacteroides, Clostridium, Eubacterium and Fusobacterium) are the predominant microflora that inhabit the lower gastrointestinal tract (facultative anaerobes: 10^4 to 10^9 cfu/g; strict anaerobes: 10^6 to 10^{10} cfu/g) of humans, with the anaerobes outnumbering facultative anaerobes (e.g., lactobacilli and streptococci) by a factor of 10^2 to 10^3 (Table IV).

Data in Table IV also show differences in microbial numbers with respect to the experimental diets. The best illustrations of differences in the microbial count of feces of subjects on the various diets can be shown by the total aerobic count of subjects 1 and 2. The total aerobic cell count decreased by a factor of 10^2 to 10^3, as the subjects were transferred from the control diet to the experimental diets (i.e., Diets A and B). However, these apparent decreases in the total aerobic count were not reflected in the lactobacilli or streptococci count (representative of aerobic organisms) of these subjects. There were also changes in the total anaerobic count of subjects 1 and 2. The magnitude (10^2 to 10^3) of these changes were similar to those of the aerobic count; however, the way in which the changes were manifested differed from the aerobic count. For example, the total anaerobic count for subject 1 increased from 10^7 cfu/g on the control diet (mess hall food) to 10^{10} cfu/g on diet A (high fat diet). When subject 1 was placed on diet B (lower fat diet), the total anaerobic fecal count decreased to $<10^8$ cfu/g. However, when subject 2 was placed on diet A, the total anaerobic count decreased from $10^{8.3}$ to $10^{7.8}$ cfu/g.

It is clear from the data in Table IV that the responses of the test subjects to the various diets followed no particular pattern or trend but showed considerable variation between diets. The differences observed between microbial counts probably reflected problems in sample handling and processing (e.g., maintenance of an appropriate anaerobic environment, homogenization, etc.). All of these factors tend to influence the composition and enumeration of the fecal microflora (Finegold et al., 1983).

Although, one of the objectives of this investigation was to determine the extent to which the diets containing fat would alter the fecal microflora, it is obvious from the data in Table IV that no obvious alteration in the fecal microbial population occurred. According to Goldin (1986), Salvage (1977) and Simon and Gorbach (1984), such microbial shifts should not be expected, since ecologic forces play a major role in defining

intestinal bacterial populations. Ecologic principles assign each species a niche that is unique to the organism (e.g., each niche is defined by host physiology, microbial interaction and environmental pressures). The microbial niche is a very complex ecosystem with high resistance to change. Thus it is believed that because of its complexity, the ecosystem established in the gut is able to rapidly reestablish itself to it original make up after being environmental stressed (e.g., dietary stress).

The data presented in this study do not in any way refute the claim that there are no significant changes in the composition of the intestinal bacterial population when the diet is altered; however, feces may not be the best samples for the study. The collection of microbes in the feces may be representative of the types of bacteria, and their proportions, but metabolic products produced by microbes in the large intestine may be more important substances for predicting microbial activity of gut inhabitants (Tannock, 1983). It must also be recognized that the current bacteriological procedures of counting the number of viable fecal bacteria are an insensitive means of monitoring the ecosystem in relation to changes in an animal environment, e.g., diet.

From the foregoing discussion, it seems perfectly reasonable to assume that the impact the NSM ration had on the gut ecosystem of the test subjects was at best inconclusive, although the conclusion that will be drawn is that the fecal microbiota was not adversely affected when test subjects were on the experimental diets. In order to make this latter statement, a different approach should have been taken. The indigenous microbiota of a particular region of gastrointestinal tract should be studied by monitoring the microflora in relation to the host's diet and environment. Specifically, it is necessary to ascertain biochemical activities of fecal bacteria of that region, e.g., β-glucuronidase activity, volatile fatty acids, hydrogen sulfide production, and pH. By knowing the biochemical activity of the ecosystem system and the biochemical potential of biotypes, it may then be feasible to show the influence of diet on the gut ecosystem.

The data presented in Table III deals with the metabolic activity of the fecal microflora of tests subjects being fed the NSM ration. While the test subjects were on the control diet (mess hall menu), the fecal microbial β-glucuronidase activity varied from 1.29 to 3.85 μg/min/mg protein, with a mean baseline value of 2.69. When the subjects were placed on diet A (high fat) the enzyme activity varied from 1.83 to 4.80 μg/min/mg protein (mean value of 3.17). When subjects were placed on diet B (lower fat), the β-glucuronidase activity ranged from 1.56 to 7.54, with a mean value of 3.78. The effect of the high-fat

diet was to increase the enzyme activity of the fecal bacteria an average of 15% over control levels ($P < 0.05$). There was also a tendency for enzyme activity to increase even when the subjects were placed on the low-fat diet (+21%). According to Reddy et al. (1980), a change from a high-fat to a low-fat (basal) diet may require a period of more than four weeks before the enzyme activity reverts to a pre-diet level.

Table V

Effect of dietary fat on human Beta-glucuronidase activity[a]

Diet A: Basal diet plus added fat Diet B: Basal diet

	Test Subject									
	1	2	3	4	5	6	7	8	Mean	SEM
Control	3.58	2.09	1.46	3.61	3.58	1.29	2.59	3.14	2.70	0.33
Diet A	nd[b]	nd	2.21	nd	4.80	1.83	nd	3.85	3.17	1.39
Diet B	2.07	2.20	1.56	4.10	7.54	2.33	4.55	4.18	3.78	2.02

[a]Enzyme activity (µg/min/mg protein)

[b]nd: analysis not done

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APPENDIX D

Individual Body Mass Changes and Endurance Exercise Times

Individual Body Mass Changes						
	Basal Diet			Basal Diet Plus Additional Fat		
Subj. No.	Pre Body Mass (kg)	Post Body Mass (kg)	Change (kg)	Pre Body Mass (kg)	Post Body Mass (kg)	Change (kg)
1	68.18	65.45	-2.73	66.68	66.23	-0.45
2	69.18	68.41	-0.77	70.00	68.64	-1.36
3	73.64	73.64	0.00	74.50	73.77	-0.73
4	73.91	72.23	-1.68	74.55	72.27	-2.27
5	92.73	90.91	-1.82	93.95	90.45	-3.50
6	79.55	76.55	-3.00	75.91	75.45	-0.45
7	97.73	94.95	-2.77	101.36	100.00	-1.36
8	91.36	87.73	-3.64	91.36	87.73	-3.64
Mean	81.12	78.73	-2.05	81.04	79.32	-1.72
SD	10.83	10.29	1.22	11.86	11.20	1.29
SEM	3.83	3.64	0.43	4.19	3.96	0.46

Body mass decreased significantly in time on both diets ($P<0.05$), but there was no difference between diets ($P>0.05$).

Treadmill Endurance Times (min)		
Subject Number	Basal Diet	Basal Plus Added Fat
1	121.67	126.50
2	112.17	109.77
3	52.07*	98.00
4	109.50	91.82
5	115.23	97.50
6	125.81	136.80
7	65.60	70.30
8	146.68	122.70
Mean	106.09	106.67
SD	31.60	21.40
SEM	11.20	7.60

*Subject stopped exercise prematurely due to a viral infection. There was no significant difference in overall endurance times with or without subject No. 3.

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